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(54) Title: MN GENE AND PROTEIN
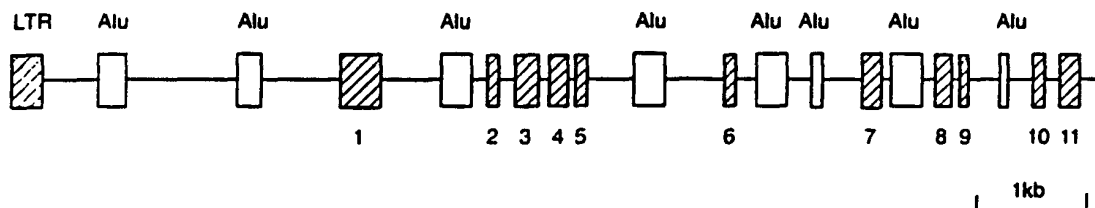
7) Abstract

A complete genomic sequence including a full-length cDNA sequence for the MN gene, a putative oncogene, is disclosed, as well proteins/polypeptides encoded thereby. Recombinant nucleic acid molecules for expressing MN proteins/polypeptides and recombinant eins are also provided. Expression of the MN gene is disclosed as being associated with tumorigenicity, and the invention concerns ods and compositions for detecting and/or quantitating MN antigen and/or MN-specific antibodies in vertebrate samples that are ostic/prognostic for neoplastic and preneoplastic disease. Test kits embodying the immunoassays f this invention are provided. MN-fic antibodies are disclosed that can be used diagnostically/prognstically, therapeutically, for imaging, and/or for affinity purification N proteins/polypeptides. Also provided are nucleic acid probes for the MN gene as well as test kits comprising said probes. The tion als c ncerns vaccines comprising MN proteins/polypeptides which are effective t immunize a vertebrate against neoplastic es associated with the expression of MN proteins. The invention still further concerns antisense nucleic acid sequences that can be o inhibit MN gene expression, and polymerase chain reaction (PCR) assays t detect genetic rearrangements in MN genes.

MN GENE AND PROTEIN
FIELD OF THE INVENTION

The present invention is in the general area of medical genetics and in the fields of biochemical engineering and immunochemistry. More specifically, it relates to the identification of a new gene--the MN gene--a cellular gene

5 coding for the MN protein. The inventors hereof found MN proteins to be associated with tumorigenicity. Evidence indicates that the MN protein appears to represent a potentially novel type of oncoprotein. Identification of MN antigen as well as antibodies specific therefor in patient

10 samples provides the basis for diagnostic/prognostic assays for cancer.

BACKGROUND OF THE INVENTION

A novel quasi-viral agent having rather unusual properties was detected by its capacity to complement mutants

15 of vesicular stomatitis virus (VSV) with heat-labile surface G protein in HeLa cells (cell line derived from human cervical adenocarcinoma), which had been cocultivated with human breast carcinoma cells. [Zavada et al., Nature New Biol., 240: 124 (1972); Zavada et al., J. Gen. Virol., 24: 327 (1974);

20 Zavada, J., Arch. Virol., 50: 1 (1976); Zavada, J., J. Gen. Virol., 63: 15-24 (1982); Zavada and Zavadova, Arch. Virol., 118: 189 (1991).] The quasi viral agent was called MaTu as it was presumably derived from a human mammary tumor.

There was significant medical interest in studying

25 and characterizing MaTu as it appeared to be an entirely new type of molecular parasite of living cells, and possibly originated from a human tumor. Zavada et al., International Publication Number WO 93/18152 (published 1 September 1993), describes the elucidation of the biological and molecular

30 nature of MaTu which resulted in the discovery of the MN gene and protein. MaTu was found by the inventors to be a two-

component system, having an exogenous transmissible component,
MX, and an endogenous cellular component, MN. The MN
component was found to be a cellular gene, showing only very
little homology with known DNA sequences. The MN gene was
5 found to be present in the chromosomal DNA of all vertebrates
tested, and its expression was found to be strongly correlated
with tumorigenicity.

The exogenous MaTu-MX transmissible agent was
identified as lymphocytic choriomeningitis virus (LCMV) which
10 persistently infects HeLa cells. The inventors discovered
that the MN expression in HeLa cells is positively regulated
by cell density, and also its expression level is increased by
persistent infection with LCMV.

Research results provided herein show that cells
15 transfected with MN cDNA undergo changes indicative of
malignant transformation. Further research findings indicate
that the disruption of cell cycle control is one of the
mechanisms by which MN may contribute to the complex process
of tumor development.

20 Described herein is the cloning and sequencing of
the MN gene and the recombinant production of MN proteins.
The full-length MN cDNA sequence [SEQ. ID. NO.: 1], the amino
acid sequence deduced therefrom [SEQ. ID. NO.: 2], a full-
length genomic sequence for MN [SEQ. ID. NO.: 5] including a
25 proposed promoter sequence [SEQ. ID. NO.: 27] are provided.
Eleven exons [SEQ. ID. NOS. 28-38] and ten introns [SEQ. ID.
NOS.: 39-48] are comprised by the MN gene. Also a 1.4
kilobase region [SEQ. ID. NO. 49] within the middle of the MN
genomic sequence is described herein, which has the character
30 of a typical CpG-rich island, and which contains multiple
putative binding sites for transcription factors AP2 and Sp1.

Also described are antibodies prepared against
proteins/polypeptides. MN proteins/ polypeptides can be used
in serological assays according to this invention to detect
35 MN-specific antibodies. Further, MN proteins/polypeptides
and/or antibodies reactive with MN antigen can be used in
immunoassays according to this invention to detect and/or

quantitate MN antigen. Such assays may be diagnostic and/or prognostic for neoplastic/pre-neoplastic disease.

SUMMARY OF THE INVENTION

This invention is directed to the MN gene, fragments thereof and the related cDNA which are useful, for example, as follows: 1) to produce MN proteins/ polypeptides by biochemical engineering; 2) to prepare nucleic acid probes to test for the presence of the MN gene in cells of a subject; 3) to prepare appropriate polymerase chain reaction (PCR) primers for use, for example, in PCR-based assays or to produce nucleic acid probes; 4) to identify MN proteins and polypeptides as well as homologs or near homologs thereto; 5) to identify various mRNAs transcribed from MN genes in various tissues and cell lines, preferably human; and 6) to identify mutations in MN genes. The invention further concerns purified and isolated DNA molecules comprising the MN gene or fragments thereof, or the related cDNA or fragments thereof.

Thus, this invention in one aspect concerns isolated nucleic acid sequences that encode MN proteins or polypeptides wherein the nucleotide sequences for said nucleic acids are selected from the group consisting of:

(a) SEQ. ID. NO.: 1;

(b) nucleotide sequences that hybridize under stringent conditions to SEQ. ID. NO.: 1 or to its complement;

(c) nucleotide sequences that differ from SEQ. ID. NO.: 1 or from the nucleotide sequences of (b) in codon sequence because of the degeneracy of the genetic code.

Further, such nucleic acid sequences are selected from nucleotide sequences that but for the degeneracy of the genetic code would hybridize to SEQ. ID. NO.: 1 or to its complement under stringent hybridization conditions.

Further, such isolated nucleic acids that encode MN proteins or polypeptides can also include the MN nucleic acids of the genomic sequence shown in Figure 3a-d, that is, SEQ. ID. NO.: 5, as well as sequences that hybridize to it or its complement under stringent conditions, or would hybridize to SEQ. ID. NO.: 5 or to its complement under such conditions,

but for the degeneracy of the genetic code. Degenerate
variants of SEQ. ID. NOS.: 1 and 5 are within the scope of
the invention.

    Further, this invention concerns nucleic acid probes
5 which are fragments of the isolated nucleic acids that encode
MN proteins or polypeptides as described above. Preferably
said nucleic acid probes are comprised of at least 29
nucleotides, more preferably of at least 50 nucleotides, still
more preferably at least 100 nucleotides, and even more
10 preferably at least 150 nucleotides.

    Still further, this invention is directed to
isolated nucleic acids containing at least twenty-seven
nucleotides selected from the group consisting of:

    (a) SEQ. ID. NOS.: 1, 5 and 27-49 and that are
15 complementary to SEQ. ID. NOS.: 1, 5 and 27-49;

    (b) nucleotide sequences that hybridize under
standard stringent hybridization conditions to one or more of
the following nucleotide sequences: SEQ. ID. NOS.: 1, 5, and
27-49 and the respective complements of SEQ. ID. NOS.: 1, 5
20 and 27-49; and

    (c) nucleotide sequences that differ from the
nucleotide sequences of (a) and (b) in codon sequence because
of the degeneracy of the genetic code. The invention also
concerns nucleic acids that but for the degeneracy of the
25 genetic code would hybridize to the nucleic acids of (a) and
(b) under standard stringent hybridization conditions.
Further this invention concerns nucleic acids of (b) and (c)
that hybridize partially or wholly to the non-coding regions
of SEQ. ID. NO.: 5 or its complement as, for example,
30 sequences that function as nucleic acid probes to identify MN
nucleic acid sequences. Conventional technology can be used
to determine whether the nucleic acids of (b) and (c) or of
fragments of SEQ. ID. NO.: 5 are useful to identify MN
nucleic acid sequences, for example, as outlined in Benton and
35 Davis, _Science, 196_: 180 (1977) and Fuscoe et al. _Genomics,_
_5_: 100 (1989). In general, such nucleic acids are preferably
at least 29 nucleotides, most preferably at least 50
nucleotides and still more preferably at least 100

nucleotides. An exemplary and preferred nucleic acid probe is SEQ. ID. NO.: 55 (a 470 bp probe useful in RNase portection assays).

Test kits of this invention can comprise the nucleic acid probes of the invention which are useful diagnostically/prognostically for neoplastic and/or pre-neoplastic disease. Preferred test kits comprise means for detecting or measuring the hybridization of said probes to the MN gene or to the mRNA product of the MN gene, such as a visualizing means.

Fragments of the isolated nucleic acids of the invention, can also be used as PCR primers to amplify segments of MN genes, and may be useful in identifying mutations in MN genes. Typically, said PCR primers are olignucleotides, preferably at least 16 nucleotides, but they may be considerably longer. Exemplary primers may be from about 16 nucleotides to about 50 nucleotides, preferably from about 19 nucleotides to about 45 nucleotides.

Further, the invention concerns the use of such PCR primers in methods to detect mutations in an isolated MN gene and/or fragment(s) thereof. For example, such methods can comprise amplifying one or more fragment(s) of an MN gene by PCR, and determining whether any of said one or more fragments contain mutations, by, for example, comparing the size of the amplified fragments to those of similarly amplified corresponding fragments of MN genes known to be normal, by using a PCR-single-strand conformation polymorphism assay or a denaturing gradient gel electrophoretic assay.

This invention also concerns nucleic acids which encode MN proteins or polypeptides that are specifically bound by monoclonal antibodies designated M75 that are produced by the hybridoma VU-M75 deposited at the American Type Culture Collection (ATCC) at 12301 Parklawn Drive in Rockville, Maryland 20852 (USA) under ATCC No. HB 11128, and/or by monoclonal antibodies designated MN12 produced by the hybridoma MN 12.2.2 deposited at the ATCC under ATCC No. HB 11647.

This invention further concerns isolated nucleic acids containing at least sixteen nucleotides, preferably at least twenty-nine nucleotides, more preferably at least fifty nucleotides, wherein said nucleic acid is selected from the

5   group consisting of:

(a) the MN nucleic acids contained in plasmids A4a, XE1 and XE3 which were deposited at the American Type Culture Collection (ATCC) in Rockville, Maryland in the United States of America under the respective ATCC Nos. 97199, 97200, and

10   97198;

(b) nucleic acids that hybridize under stringent conditions to the MN nucleic acids of (a); and

(c) nucleic acids that differ from the nucleic acids of (a) or (b) in codon sequence due to the degeneracy of the

15   genetic code. Such isolated nucleic acids, for example, can be polymerase chain reaction (PCR) primers.

The invention further concerns isolated nucleic acids that code for an MN protein, MN fusion protein or MN polypeptide that is operatively linked to an expression

20   control sequence within a vector; unicellular hosts, prokaryotic or eukaryotic, that are transformed or transfected therewith; and methods of recombinantly producing MN proteins, MN fusion proteins and MN polypeptides comprising transforming or transfecting unicellular hosts with said nucleic acid

25   operatively linked to an expression control sequence, culturing said transformed or transfected unicellular hosts so that said MN proteins, fusion proteins or polypeptides are expressed, and extracting and isolating said MN protein fusion protein or polypeptide.

30   Recombinant nucleic acids that encode MN fusion proteins are claimed as consisting essentially of an MN protein or MN polypeptide and a non-MN protein or polypeptide wherein the nucleotide sequence for the portion of the nucleic acid encoding the MN protein or polypeptide is selected from

35   the group consisting of:

(a) SEQ. ID. NO.: 1;

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(b) nucleotide sequences that hybridize under stringent conditions to SEQ. ID. NO.: 1 or to its complement; and

5 (c) degenerate variants of SEQ. ID. NO.: 1, and of the nucleotide sequences of (b);

wherein the nucleic acid encoding said MN protein or polypeptide contains at least twenty-nine nucleotides.

Said non-MN protein or polypeptide may preferably be nonimmunogenic to humans and not typically reactive to 10 antibodies in human body fluids. Examples of such a DNA sequence is the alpha-peptide coding region of beta-galactosidase and a sequence coding for glutathione S-transferase or a fragment thereof. However, in some instances, a non-MN protein or polypeptide that is 15 serologically active, immunogenic and/or antigenic may be preferred as a fusion partner to a MN antigen. Further, claimed herein are such recombinant fusion proteins/polypeptides which are substantially pure and non-naturally occurring. Exemplary fusion proteins of this invention are 20 GEX-3X-MN, MN-Fc and MN-PA, described infra.

In HeLa and in tumorigenic HeLa x fibroblast hybrid (H/F-T) cells, MN protein is manifested as a "twin" protein p54/58N; it is glycosylated and forms disulfide-linked oligomers. As determined by electrophoresis upon reducing 25 gels, MN proteins have molecular weights in the range of from about 40 kd to about 70 kd, preferably from about 48 kd to about 58 kd. about 65 kd, more preferably from about 45 kd to Upon non-reducing gels, MN proteins in the form of oligomers have molecular weights in the range of from about 145 kd to 30 about 160 kd, preferably from about 150 to about 155 kd, still more preferably from about 152 to about 154 kd. A predicted amino acid sequence for a preferred MN protein of this invention is shown in Figure 1 [SEQ. ID. NO. 2].

Other particular MN proteins or polypeptides are 35 exemplified by the putative MN signal peptide shown as the first thirty-seven amino acids in Figure 1 [SEQ. ID. NO.: 6], preferred MN antigen epitopes [SEQ. ID. NOS.: 10-16], and domains of the MN protein represented in Figure 1 as amino

acids 38-135 [SEQ. IS. NO.: 50], 136-391 [SEQ. ID. NO.: 51],
414-433 [SEQ. ID. NO.: 52], and 434-459 [SEQ. ID. NO.: 53].

       The discovery of the MN gene and protein and thus,
of substantially complementary MN genes and proteins encoded

5   thereby, led to the finding that the expression of MN proteins
was associated with tumorigenicity. That finding resulted in
the creation of methods that are diagnostic/ prognostic for
cancer and precancerous conditions. Methods and compositions
are provided for identifying the onset and presence of

10 neoplastic disease by detecting and/or quantitating MN antigen
in patient samples, including tissue sections and smears, cell
and tissue extracts from vertebrates, preferably mammals and
more preferably humans. Such MN antigen may also be found in
body fluids.

15   MN proteins and genes are of use in research
concerning the molecular mechanisms of oncogenesis, in cancer
diagnostics/prognostics, and may be of use in cancer
immunotherapy. The present invention is useful for detecting
a wide variety of neoplastic and/or pre-neoplastic diseases.

20 Exemplary neoplastic diseases include carcinomas, such as
mammary, bladder, ovarian, uterine, cervical, endometrial,
squamous cell and adenosquamous carcinomas; and head and neck
cancers; mesodermal tumors, such as neuroblastomas and
retinoblastomas; sarcomas, such as osteosarcomas and Ewing's

25 sarcoma; and melanomas. Of particular interest are head and
neck cancers, gynecologic cancers including ovarian, cervical,
vaginal, endometrial and vulval cancers; gastrointestinal
cancer, such as, stomach, colon and esophageal cancers;
urinary tract cancer, such as, bladder and kidney cancers;

30 skin cancer; liver cancer; prostate cancer; lung cancer; and
breast cancer. Of still further particular interest are
gynecologic cancers; breast cancer; urinary tract cancers,
especially bladder cancer; lung cancer; and liver cancer.
Even further of particular interest are gynecologic cancers

35 and breast cancer. Gynecologic cancers of particular interest
are carcinomas of the uterine cervix, endometrium and ovaries;
more particularly such gynecologic cancers include cervical
squamous cell carcinomas, adenosquamous carcinomas,

adenocarcinomas as well as gynecologic precancerous conditions, such as metaplastic cervical tissues and condylomas.

5   The invention further relates to the biochemical engineering of the MN gene, fragments thereof or related cDNA. For example, said gene or a fragment thereof or related cDNA can be inserted into a suitable expression vector, wherein it is operatively linked to an expression control sequence; host cells, preferably unicellular, can be transformed or

10   transfected with such an expression vector; and an MN protein/polypeptide, preferably an MN protein, is expressed therein. Such a recombinant protein or polypeptide can be glycosylated or nonglycosylated, preferably glycosylated, and can be purified to substantial purity. The invention further

15   concerns MN proteins/polypeptides which are synthetically or otherwise biologically prepared.

         Said MN proteins/polypeptides can be used in assays to detect MN antigen in patient samples and in serological assays to test for MN-specific antibodies. MN

20   proteins/polypeptides of this invention are serologically active, immunogenic and/or antigenic. They can further be used as immunogens to produce MN-specific antibodies, polyclonal and/or monoclonal, as well as an immune T-cell response.

25   The invention further is directed to MN-specific antibodies, which can be used diagnostically/prognostically and may be used therapeutically. Preferred according to this invention are MN-specific antibodies reactive with the epitopes represented respectively by the amino acid sequences

30   of the MN protein shown in Figure 1 as follows: from AA 62 to AA 67 [SEQ. ID. NO.: 10]; from AA 55 to AA 60 [SEQ. ID. NO.: 11]; from AA 127 to AA 147 [SEQ. ID. NO.: 12]; from AA 36 to AA 51 [SEQ. ID. NO.: 13]; from AA 68 to AA 91 [SEQ. ID. NO.: 14]; from AA 279 to AA 291 [SEQ. ID. NO.: 15]; and from AA

35   435 to AA 450 [SEQ. ID. NO.: 16]. More preferred are antibodies reactive with epitopes represented by SEQ. ID. NOS.: 10, 11 and 12. Still more preferred are antibodies reactive with the epitopes represented by SEQ. ID NOS: 10 and

11, as for example, respectively Mabs M75 and MN12. Most preferred are monoclonal antibodies reactive with the epitope represented by SEQ. ID. NO.: 10.

Also preferred according to this invention are
5 antibodies prepared against recombinantly produced MN proteins as, for example, GEX-3X-MN, MN 20-19, MN-Fc and MN-PA. Also preferred are MN-specific antibodies prepared against glycosylated MN proteins, such as, MN 20-19 expressed in baculovirus infected Sf9 cells.

10 A hybridoma that produces a representative MN-specific antibody, the monoclonal antibody M75 (Mab M75), was deposited at the under ATCC Number HB 11128 as indicated above. The M75 antibody was used to discover and identify the MN protein and can be used in radioimmunoassays and immunohistochemically,
15 Western blots, in tissue samples that are fresh, frozen, or for example, formalin-, alcohol-, acetone- or otherwise fixed and/or paraffin-embedded and deparaffinized. Another representative MN-specific antibody, Mab MN12, is secreted by the hybridoma
20 MN 12.2.2, which was deposited at the ATCC under the designation HB 11647.

MN-specific antibodies can be used, for example, in laboratory diagnostics, using immunofluorescence microscopy or immunohistochemical staining; as a component in immunoassays
25 for detecting and/or quantitating MN antigen in, for example, clinical samples; as probes for immunoblotting to detect MN antigen; in immunoelectron microscopy with colloid gold beads for localization of MN proteins and/or polypeptides in cells; and in genetic engineering for cloning the MN gene or
30 fragments thereof, or related cDNA. Such MN-specific antibodies can be used as components of diagnostic/prognostic kits, for example, for in vitro use on histological/prognostic such antibodies can also and used for in vivo diagnostics/ prognostics, for example, such antibodies can be labeled
35 appropriately, as with a suitable radioactive isotope, and used in vivo to locate metastases by scintigraphy. Further such antibodies may be used in vivo therapeutically to treat cancer patients with or without toxic and/or cytostatic agents

attached thereto. Further, such antibodies can be used <u>in
vivo</u> to detect the presence of neoplastic and/or pre-
neoplastic disease. Still further, such antibodies can be
used to affinity purify MN proteins and polypeptides.

5   This invention also concerns methods of treating
neoplastic disease and/or pre-neoplastic disease comprising
inhibiting the expression of MN genes by administering
antisense nucleic acid sequences that are substantially
complementary to mRNA transcribed from MN genes. Said

10   antisense nucleic acid sequences are those that hybridize to
such mRNA under stringent hybridization conditions. Preferred
are antisense nucleic acid sequences that are substantially
complementary to sequences at the 5' end of the MN cDNA
sequence shown in Figure 1. Preferably said antisense nucleic

15   acid sequences are oligonucleotides.

           This invention also concerns vaccines comprising an
immunogenic amount of one or more substantially pure MN
proteins and/or polypeptides dispersed in a physiologically
acceptable, nontoxic vehicle, which amount is effective to

20   immunize a vertebrate, preferably a mammal, more preferably a
human, against a neoplastic disease associated with the
expression of MN proteins. Said proteins can be
recombinantly, synthetically or otherwise biologically
produced. A particular use of said vaccine would be to

25   prevent recidivism and/or metastasis. For example, it could
be administered to a patient who has had an MN-carrying tumor
surgically removed, to prevent recurrence of the tumor.

           The immunoassays of this invention can be embodied
in test kits which comprise MN proteins/polypeptides and/or

30   MN-specific antibodies. Such test kits can be in solid phase
formats, but are not limited thereto, and can also be in
liquid phase format, and can be based on immunohistochemical
assays, ELISAS, particle assays, radiometric or fluorometric
assays either unamplified or amplified, using, for example,

35   avidin/biotin technology.

                            <u>Abbreviations</u>
           The following abbreviations are used herein:

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AA	-	amino acid
ATCC	-	American Type Culture Collection
bp	-	base pairs
BLV	-	bovine leukemia virus
5 BSA	-	bovine serum albumin
BRL	-	Bethesda Research Laboratories
CA	-	carbonic anhydrase
CAT	-	chloramphenicol acetyltransferase
Ci	-	curie
10 cm	-	centimeter
CMV	-	cytomegalovirus
cpm	-	counts per minute
C-terminus	-	carboxyl-terminus
°C	-	degrees centigrade
15 DEAE	-	diethylaminoethyl
DMEM	-	Dulbecco modified Eagle medium
EDTA	-	ethylenediaminetetraacetate
EIA	-	enzyme immunoassay
ELISA	-	enzyme-linked immunosorbent assay
20 F	-	fibroblasts
FCS	-	fetal calf serum
FITC	-	fluorescein isothiocyanate
GEX-3X-MN	-	fusion protein MN glutathione S-transferase
H	-	HeLa cells
25 HEF	-	human embryo fibroblasts
HeLa K	-	standard type of HeLa cells
HeLa S	-	Stanbridge's mutant HeLa D98/AH.2
H/F-T	-	hybrid HeLa fibroblast cells that are tumorigenic; derived from HeLa D98/AH.2
30 H/F-N	-	hybrid HeLa fibroblast cells that are nontumorigenic; derived from HeLa D98/AH.2
HRP	-	horseradish peroxidase
Inr	-	initiator
IPTG	-	isopropyl-Beta-D-thiogalacto-pyranoside
35 kb	-	kilobase
kbp	-	kilobase pairs
kd	-	kilodaltons
LCMV	-	lymphocytic choriomeningitis virus

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	LTR	-	long terminal repeat
	M	-	molar
	mA	-	milliampere
	MAb	-	monoclonal antibody
5	ME	-	mercaptoethanol
	MEM	-	minimal essential medium
	min.	-	minute(s)
	mg	-	milligram
	ml	-	milliliter
10	mM	-	millimolar
	MMC	-	mitomycin C
	MLV	-	murine leukemia virus
	N	-	normal concentration
	NEG	-	negative
15	ng	-	nanogram
	nt	-	nucleotide
	N-terminus	-	amino-terminus
	ODN	-	oligodeoxynucleotide
	ORF	-	open reading frame
20	PA	-	Protein A
	PBS	-	phosphate buffered saline
	PCR	-	polymerase chain reaction
	PEST	-	combination of one-letter abbreviations for proline, glutamic acid, serine, threonine
25	pI	-	isoelectric point
	PMA	-	phorbol 12-myristate 13-acetate
	POS	-	positive .
	Py	-	pyrimidine
	RIA	-	radioimmunoassay
30	RIP	-	radioimmunoprecipitation
	RIPA	-	radioimmunoprecipitation assay
	RNP	-	RNase protection assay
	SDRE	-	serum dose response element
	SDS	-	sodium dodecyl sulfate
35	SDS-PAGE	-	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
	SINE	-	short interspersed repeated sequence
	SSDS	-	synthetic splice donor site

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SP-RIA	-	solid-phase radioimmunoassay
SSDS	-	synthetic splice donor site
SSPE	-	NaCl (0.18 M), sodium phosphate (0.01 M), EDTA (0.001 M)
TBE	-	Tris-borate/EDTA electrophoresis buffer
TCA	-	trichloroacetic acid
TC media	-	tissue culture media
TMB	-	tetramethylbenzidine
Tris	-	tris (hydroxymethyl) aminomethane
μCi	-	microcurie
μg	-	microgram
μl	-	microliter
μM	-	micromolar
VSV	-	vesicular stomatitis virus
X-MLV	-	xenotropic murine leukemia virus

Cell Lines

HeLa K   -- standard type of HeLa cells; aneuploid, epithelial-like cell line isolated from a human cervical adenocarcinoma [Gey et al., Cancer Res., 12: 264 (1952); Jones et al., Obstet. Gynecol., 38: 945-949 (1971)] obtained from Professor B. Korych, [Institute of Medical Microbiology and Immunology, Charles University; Prague, Czech Republic]

HeLa D98/AH.2 -- Mutant HeLa clone that is hypoxanthine
(also HeLa S)   guanine phosphoribosyl transferase-deficient (HGPRT⁻) kindly provided by Eric J. Stanbridge [Department of Microbiology, College of Medicine, University of California, Irvine, CA (USA)] and reported in Stanbridge et al., Science, 215: 252-259 (15 Jan. 1982); parent of hybrid cells H/F-N and H/F-T, also obtained from E.J. Stanbridge.

NIH-3T3   -- murine fibroblast cell line reported in Aaronson, Science, 237: 178 (1987).

XC   -- cells derived from a rat rhabdomyosarcoma induced with Rous sarcoma virus-induced rat sarcoma [Svoboda, J., Natl. Cancer Center Institute Monograph No. 17, IN: "International Conference on Avian Tumor Viruses" (J.W. Beard ed.), pp. 277-298 (1964)], kindly provided by Jan Svoboda [Institute of Molecular Genetics, Czechoslovak Academy of Sciences; Prague, Czech Republic]; and

CGL1   -- H/F-N hybrid cells (HeLa D98/AH.2 derivative)

CGL2   -- H/F-N hybrid cells (HeLa D98/AH.2 derivative)

CGL3   -- H/F-T hybrid cells (HeLa D98/AH.2 derivative)

CGL4   -- H/F-T hybrid cells (HeLa D98/Ah.2 derivative)

Nucleotide and Amino Acid Sequence Symbols

The following symbols are used to represent nucleotides herein:

Base Symbol	Meaning
A	adenine
C	cytosine
G	guanine
T	thymine
U	uracil
I	inosine
M	A or C
R	A or G
W	A or T/U
S	C or G
Y	C or T/U
K	G or T/U
V	A or C or G
H	A or C or T/U

	D		A or G or T/U
	B		C or G or T/U
	N/X		A or C or G or T/U

There are twenty main amino acids, each of which is
5 specified by a different arrangement of three adjacent
nucleotides (triplet code or codon), and which are linked
together in a specific order to form a characteristic protein.
A three-letter or one-letter convention is used herein to
identify said amino acids, as, for example, in Figure 1 as
10 follows:

Amino acid name	3 Ltr. Abbrev.	1 Ltr. Abbrev.
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Unknown or other		X

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BRIEF DESCRIPTION OF THE FIGURES

35 Figure 1 provides the nucleotide sequence for a
full-length MN cDNA [SEQ. ID. NO.: 1] clone isolated as

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described herein. Figure 1 also sets forth the predicted
amino acid sequence [SEQ. ID. NO.: 2] encoded by the cDNA.

        Figure 2 compares the results of immunizing baby
rats to XC tumor cells with rat serum prepared against the
5   fusion protein MN glutathione S-transferase (GEX-3X-MN) (the
IM group) with the results of immunizing baby rats with
control rat sera (the C group). Each point on the graph
represents the tumor weight of a tumor from one rat. Example
2 details those experiments.

10   Figure 3a-d provides a 10,898 bp complete genomic
sequence of MN [SEQ. ID. NO.: 5]. The base count is as
follows: 2654 A; 2739 C; 2645 G; and 2859 T. The 11 exons
are shown in capital letters.

        Figure 4 is a restriction map of the full-length MN
15   cDNA. The open reading frame is shown as an open box. The
thick lines below the restriction map illustrate the sizes and
positions of two overlapping cDNA clones. The horizontal
arrows indicate the positions of primers R1 [SEQ. ID. NO.: 7]
and R2 [SEQ. ID. NO.: 8] used for the 5' end RACE. Relevant
20   restriction sites are BamHI (B), EcoRV (V), EcoRI (E), PstI
(Ps), PvuII (Pv).

        Figure 5 is a map of the human MN gene. The
numbered cross-hatched boxes represent exons. The box
designated LTR denotes a region of homology to HERV-K LTR.
25   The empty boxes are Alu-related sequences.

        Figure 6 is a nucleotide sequence for the proposed
promoter of the human MN gene [SEQ. ID. No.: 27]. The
nucleotides are numbered from the transcription initiation
site according to RNase protection assay. Potential
30   regulatory elements are overlined. Transcription start sites
are indicated by asterisks (RNase protection) and dots (RACE).
The sequence of the 1st exon begins under the asterisks.

        Figure 7 provides a schematic of the alignment of MN
genomic clones according to their position related to the
35   transcription initiation site. All the genomic fragments
except Bd3 were isolated from a lambda FIX III genomic library
derived from HeLa cells. Clone Bd3 was derived from a human
fetal brain library.

Figure 8 shows the construction and cloning of a series of 5' deletion mutants of MN's putative promoter region linked to the bacterial CAT gene.

DETAILED DESCRIPTION

The MN gene is shown herein to be organized into 11 exons and 10 introns. Described herein is the cloning and sequencing of the MN cDNA and genomic sequences, and the genetic engineering of MN proteins -- such as the GEX-3X-MN, MN-PA, MN-Fc and MN 20-19 proteins. The recombinant MN proteins can be conveniently purified by affinity chromatography.

MN is manifested in HeLa cells by a twin protein, p54/58N. Immunoblots using a monoclonal antibody reactive with p54/58N (MAb M75) revealed two bands at 54 kd and 58 kd. Those two bands may correspond to one type of protein that differs by glycosylation pattern or by how it is processed. Herein, the phrase "twin protein" indicates p54/58N.

The expression of MN proteins appears to be diagnostic/prognostic for neoplastic disease. The MN twin protein, p54/58N, was found to be expressed in HeLa cells and in Stanbridge's tumorigenic (H/F-T) hybrid cells [Stanbridge et al., _Somatic Cell Genet. 7_: 699-712 (1981); and Stanbridge et al., _Science, 215_: 252-259 (1982)] but not in fibroblasts or in non-tumorigenic (H/F-N) hybrid cells [Stanbridge et al., _id._]. In early studies reported in Zavada et al. WO 93/18152, _supra_, MN proteins were found in immunoblots prepared from human ovarian, endometrial and uterine cervical carcinomas, and in some benign neoplasias (as mammary papilloma) but not from normal ovarian, endometrial, uterine or placental tissues. Example 1 herein details further research on MN gene expression wherein MN antigen, as detected by immunohistochemical staining, was found to be prevalent in tumor cells of a number of cancers, including cervical, bladder, head and neck, and renal cell carcinomas among others. Further, the immunohistochemical staining experiments of Example 1 show that among normal tissues tested, only normal stomach tissues showed routinely and extensively the

presence of MN antigen. MN antigen is further shown herein to
be present sometimes in morphologically normal-appearing areas
of tissue specimens exhibiting dysplasia and/or malignancy.

MN Gene--Cloning and Sequencing

5   Figure 1 provides the nucleotide sequence for a
full-length MN cDNA clone isolated as described below [SEQ.
ID. NO.: 1]. Figure 3a-d provides a complete MN genomic
sequence [SEQ. ID. NO.: 5]. Figure 6 shows the nucleotide
sequence for a proposed MN promoter [SEQ. ID. NO.: 27].

10   It is understood that because of the degeneracy of
the genetic code, that is, that more than one codon will code
for one amino acid [for example, the codons TTA, TTG, CTT,
CTC, CTA and CTG each code for the amino acid leucine (leu)],
that variations of the nucleotide sequences in, for example,

15   SEQ. ID. NOS.: 1 and 5 wherein one codon is substituted for
another, would produce a substantially equivalent protein or
polypeptide according to this invention. All such variations
in the nucleotide sequences of the MN cDNA and complementary
nucleic acid sequences are included within the scope of this

20   invention.
           It is further understood that the nucleotide
sequences herein described and shown in Figures 1, 3a-d and 6,
represent only the precise structures of the cDNA, genomic and
promoter nucleotide sequences isolated and described herein.

25   It is expected that slightly modified nucleotide sequences
will be found or can be modified by techniques known in the
art to code for substantially similar or homologous MN
proteins and polypeptides, for example, those having similar
epitopes, and such nucleotide sequences and proteins/

30   polypeptides are considered to be equivalents for the purpose
of this invention. DNA or RNA having equivalent codons is
considered within the scope of the invention, as are synthetic
nucleic acid sequences that encode proteins/polypeptides
homologous or substantially homologous to MN

35   proteins/polypeptides, as well as those nucleic acid sequences
that would hybridize to said exemplary sequences [SEQ. ID.
NOS. 1, 5 and 27] under stringent conditions, or that, but for

the degeneracy of the genetic code would hybridize to said
cDNA nucleotide sequences under stringent hybridization
conditions. Modifications and variations of nucleic acid
sequences as indicated herein are considered to result in
5   sequences that are substantially the same as the exemplary MN
sequences and fragments thereof.

Partial cDNA clone

        In Zavada et al., id., the isolation of a partial MN
cDNA clone of 1397 bp in length was described. A lambda gt11
10 cDNA library of LMCV-infected HeLa cells was prepared and
subjected to immunoscreening with Mab M75 in combination with
goat anti-mouse antibodies conjugated with alkaline
phosphatase. One positive clone was picked and subcloned into
the NotI site of pBlusecript KS [Stratagen; La Jolla, CA
15 (USA)] thereby creating pBluscript-MN.
        Two oppositely oriented nested deletions were made
using Erase-a-Base™ kit [Promega; Madison, WI (USA)] and
sequenced by dideoxy method with a T7 sequencing kit
[Pharmacia; Piscataway, NJ (USA)]. The sequencing showed a
20 partial cDNA clone, the insert being 1397 bp long. The
sequence comprises a large 1290 bp open reading frame and 107
bp 3' untranslated region containing a polyadenylation signal
(AATAAA). However, the sequence surrounding the first ATG
codon in the open reading frame (ORF) did not fit the
25 definition of a translational start site. In addition, as
followed from a comparison of the size of the MN clone with
that of the corresponding mRNA in a Northern blot, the cDNA
was shown to be missing about 100 bp from the 5' end of its
sequence.

30 Full-Length cDNA Clone

        Attempts to isolate a full-length clone from the
original cDNA library failed. Therefore, the inventors
performed a rapid amplification of cDNA ends (RACE) using MN-
specific primers, R1 and R2 [SEQ. ID. NOS.:   7 and 8], derived
35 from the 5' region of the original cDNA clone. The RACE
product was inserted into pBluescript, and the entire

population of recombinant plasmids was sequenced with an MN-
specific primer ODN1 [SEQ. ID. NO.: 3]. In that way, a
reliable sequence at the very 5' end of the MN cDNA as shown
in Figure 1 [SEQ. ID. NO.: 1] was obtained.

5   Specifically, RACE was performed using 5' RACE
System [GIBCO BRL; Gaithersburg, MD (USA)] as follows. 1 μg
of mRNA (the same as above) was used as a template for the
first strand cDNA synthesis which was primed by the MN-
specific antisense oligonucleotide, R1 (5'-
10 TGGGGTTCTTGAGGATCTCCAGGAG-3') [SEQ. ID. NO.: 7]. The first
strand product was precipitated twice in the presence of
ammonium acetate and a homopolymeric C tail was attached to
its 3' end by TdT. Tailed cDNA was then amplified by PCR
using a nested primer, R2 (5'-CTCTAACTTCAGGGAGCCCTCTTCTT-3')
15 [SEQ. ID. NO.: 8] and an anchor primer that anneals to the
homopolymeric tail (5'-CUACUACUACUAGGCCACGCGTCGACTAGTACGGGI
IGGGIIGGGIIG-3') [SEQ. ID. NO.: 9]. The amplified product
was digested with BamHI and SalI restriction enzymes and
cloned into pBluescript II KS plasmid. After transformation,
20 plasmid DNA was purified from the whole population of
transformed cells and used as a template for sequencing with
the MN-specific primer ODN1 [SEQ. ID. NO.: 3; a 29-mer 5'
CGCCCAGTGGGTCATCTTCCCCAGAAGAG 3'].

          Based upon results of the RACE analysis, the full-
25 length MN cDNA sequence was seen to contain a single ORF
starting at position 12, with an ATG codon that is in a good
context (GCGCATGG) with the rule proposed for translation
initiation [Kozak, J. Cell. Biol., 108: 229-241 (1989)].
[See below under Mapping of MN Gene Transcription Initiation
30 Site for fine mapping of the 5' end of the MN gene.] The AT
rich 3' untranslated region contains a polyadenylation signal
(AATAAA) preceding the end of the cDNA by 10 bp.
Surprisingly, the sequence from the original clone as well as
from four additional clones obtained from the same cDNA
35 library did not reveal any poly(A) tail. Moreover, just
downstream of the poly(A) signal, an ATTTA motif that is
thought to contribute to mRNA instability [Shaw and Kamen,
Cell, 46: 659-667 (1986)] was found. That fact raised the

possibility that the poly (A) tail is missing due to the
specific degradation of the MN mRNA.

Genomic clones

         To study MN regulation, MN genomic clones were
5   isolated. One MN genomic clone (Bd3) was isolated from a
human cosmid library prepared from fetal brain using both MN
cDNA as a probe and the MN-specific primers derived from the
5' end of the cDNA ODN1 [SEQ. ID. NO.: 3, supra] and ODN2
[SEQ. ID NO.: 4; 19-mer (5' GGAATCCTCCTGCATCCGG 3')].
10   Sequence analysis revealed that that genomic clone covered a
region upstream from a MN transcription start site and ending
with the BamHI restriction site localized inside the MN cDNA.
Other MN genomic clones can be similarly isolated.
         In order to identify the complete genomic region of
15   MN, the human genomic library in Lambda FIX II vector
(Stratagene) was prepared from HeLa chromosomal DNA and
screened by plaque hybridization using MN cDNA as described
below. Several independent MN recombinant phages were
identified, isolated and characterized by restriction mapping
20   and hybridization analyses. Four overlapping recombinants
covering the whole genomic region of MN were selected,
digested and subcloned into pBluescript. The subclones were
then subjected to bidirectional nested deletions and
sequencing. DNA sequences were compiled and analyzed by
25   computer using the DNASIS software package.
         The details of isolating genomic clones covering the
complete genomic region for MN are provided below. Figure 7
provides a schematic of the alignment of MN genomic clones
according to the transcription initiation site. Plasmids
30   containing the A4a clone and the XE1 and XE3 subclones were
deposited at the American Type Culture Collection (ATCC) at
12301 Parklawn Drive, Rockville, MD 20852 (USA) on June 6,
1995, respectively under ATCC Deposit Nos. 97199, 97200, and
97198.

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Isolation of Genomic DNA Clones

The Sau3AI human HeLa genomic library was prepared
in Lambda FIX II vector [Stratagene; La Jolla, CA (USA)]
according to manufacturer's protocol. Human fetal brain
5   cosmid library in SuperCos cosmid was from Stratagene.
Recombinant phages or bacteria were plated at 1 x 10^5 plaque
forming units on 22x22 cm Nunc plates or 5 x 10^4 cells on 150
mm Petri dishes, and plaques or colonies were transferred to
Hybond N membranes (Amersham). Hybridization was carried out
10 with the full-length MN cDNA labeled with [P^{32}]PdCTP by the
Multiprime DNA labeling method (Amersham) at 65°C in 6 x SSC,
0.5% SDS, 10 x Denhardt's and 0.2 mg/1 ml salmon sperm DNA.
Filters were washed twice in 2 x SSC, 0.1% SDS at 65°C for 20
min. The dried filters were exposed to X-ray films, and
15 positive clones were picked up. Phages and bacteria were
isolated by 3-4 sequential rounds of screening.

Subcloning and DNA Sequencing

Genomic DNA fragments were subcloned into a
pBluescript KS and templates for sequencing were generated by
20 serial nested deletions using the Erase-a-Base system.
Sequencing was performed by the dideoxynucleotide chain
termination method using T7 sequencing kit (Pharmacia).
Nucleotide sequence alignments and analyses were carried out
using the DNASIS software package (Hitachi Software
25 Engineering).

Exon-Intron Structure of Complete MN Genomic Region

The complete sequence of the overlapping clones
contains 10,898 bp (SEQ. ID. NO.:   5). Figure 5 depicts the
organization of the human MN gene, showing the location of all
30 11 exons as well as the 2 upstream and 6 intronic Alu repeat
elements. All the exons are small, ranging from 27 to 191 bp,
with the exception of the first exon which is 415 bp. The
intron sizes range from 89 to 1400 bp.
Table 1 below lists the splice donor and acceptor
35 sequences that conform to conser.sus splice sequences including

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the AG-GT motif [Mount, "A catalogue of splice junction sequences," <u>Nucleic Acids Res. 10</u>: 459-472 (1982)].

TABLE 1
Exon-Intron Structure of the Human MN Gene

Exon	Size	Genomic Position**	SEQ ID NO	5'splice donor	SEQ ID No
1	415	*3537-3951	28	AGAAG gtaagt	67
2	30	5126-5155	29	TGGAG gtgaga	68
3	171	5349-5519	30	CAGTC gtgagg	69
4	143	5651-5793	31	CCGAG gtgagc	70
5	93	5883-5975	32	TGGAG gtacca	71
6	67	7376-7442	33	GGAAG gtcagt	72
7	158	8777-8934	34	AGCAG gtgggc	73
8	145	9447-9591	35	GCCAG gtacag	74
9	27	9706-9732	36	TGCTG gtgagt	75
10	82	10350-10431	37	CACAG gtatta	76
11	191	10562-10752	38	ATAAT end	

Intron	Size	Genomic Position**	SEQ ID NO	3'splice acceptor	SEQ ID NO
1	1174	3952-5125	39	atacag GGGAT	77
2	193	5156-5348	40	ccccag GCGAC	78
3	131	5520-5650	41	acgcag TGCAA	79
4	89	5794-5882	42	tttcag ATCCA	80
5	1400	5976-7375	43	ccccag GAGGG	81
6	1334	7443-8776	44	tcacag GCTCA	82
7	512	8935-9446	45	ccctag CTCCA	83
8	114	9592-9705	46	ctccag TCCAG	84
9	617	9733-10349	47	tcgcag GTGACA	85
10	130	10432-10561	48	acacag AAGGG	86

** positions are related to nt numbering in whole genomic sequence including the 5' flanking region [Figure 3a-d]

* number corresponds to transcription initiation site determined below by RNase protection assay

A search for sequences related to MN gene in the EMBL Data Library did not reveal any specific homology except for 6 complete and 2 partial Alu-type repeats with homology to Alu sequences ranging from 69.8% to 91% [Jurka and
5 Milosavljevic, "Reconstruction and analysis of human Alu genes," J. Mol. Evol. 32: 105-121 (1991)]. Below under the Characterization of the 5' Flanking Region, also a 222 bp sequence proximal to the 5' end of the genomic region is shown to be closely homologous to a region of the HERV-K LTR.

10 Mapping of MN Gene Transcription Initiation Site
In the earlier attempt to localize the site of transcription initiation of the MN gene by RACE (above), the obtained a major PCR fragment whose sequence placed the start site 12 bp upstream from the first codon of the ORF. That
15 result was obtained probably due to a preferential amplification of the shortest form of mRNA. Therefore, the inventors used an RNase protection assay (RNP) for fine mapping of the 5' end of the MN gene. The probe was a uniformly labeled 470 nucleotide copy RNA (nt -205 to +265)
20 [SEQ. ID. NO.: 55], which was hybridized to total RNA from MN-expressing HeLa and CGL3 cells and analyzed on a sequencing gel. That analysis has shown that the MN gene transcription initiates at multiple sites, the 5' end of the longest MN transcript being 30 nt longer than that previously
25 characterized by RACE.

RNase Protection Assay
^{32}P-labeled RNA probes were prepared with an RNA Transcription kit (Stratagene). In vitro transcription reactions were carried out using 1 μg of the linearized
30 plasmid as a template, 50 μCi of [P^{32}P]rUTP (800 Ci/mmol), 10 U of either T3 or T7 RNA polymerase and other components of the Transcription Kit following instructions of the supplier. For mapping of the 5' end of MN mRNA, the 470 bp NcoI-BamHI fragment (NcoI filled in by Klenow enzyme) of Bd3 clone (nt -
35 205 to +265 related to transcription start) was subcloned to EcoRV-BamHI sites of pBluescript SK+, linearized with HindIII

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and labeled with T3 RNA polymerase. For the 3' end mRNA analysis, probe, that was prepared using T7 RNA polymerase on KS-dXE3-16 template (one of the nested deletion clones of MN genomic region XE3 subclone) digested with Sau3AI (which cuts exon 11 at position 10,629), was used. Approximately 3 x 10⁵
5 cpm of RNA probe were used per one RNase protection assay reaction.

RNase protection assays (RNP) were performed using Lysate RNase Protection Kit (USB/Amersham) according to protocols of the supplier. Briefly, cells were lysed using
10 Lysis Solution at concentration of approximately 10⁷ cells/ml, and 45 μl of the cell homogenate were used in RNA/RNA hybridization reactions with ³²p-labeled RNA probes prepared as described above. Following overnight hybridizations at 42°C, homogenates were treated for 30 min at 37°C with RNase cocktail
15 mix. Protected RNA duplexes were run on polyacrylamide/urea denaturing sequencing gels. Fixed and dried gels were exposed to X-ray film for 24 - 72 hours.

Mapping of MN Gene Transcription Termination Site

An RNase protection assay, as described above, was
20 also used to verify also the 3' end of the MN cDNA. That was important with respect to our previous finding that the cDNA contains a poly(A) signal but lacks a poly(A) tail, which could be lost during the proposed degradation of MN mRNA due to the presence of an instability motif in its 3' untranslated
25 region. RNP analysis of MN mRNA with the fragment of the genomic clone XE3 covering the region of interest corroborated our data from MN cDNA sequencing, since the 3' end of the protected fragment corresponded to the last base of MN cDNA (position 10,752 of the genomic sequence). That site also
30 meets the requirement for the presence of a second signal that is needed for transcription termination and polyadenylation [McLauchlan et al., *Nucleic Acids Res., 13*: 1347 (1985)]. Motif TGTGTTAGT (nt 10,759-
35 10,767) corresponds well to both the consensus sequence and the position of that signal within 22 bp downstream from the polyA signal (nt 10,737-10,742).

Characterization of the 5' Flanking Region

The Bd3 genomic clone isolated from human fetal brain cosmid library was found to cover a region of 3.5 kb upstream from the transcription start site of the MN gene. It contains no significant coding region. Two Alu repeats are situated at positions -2587 to -2296 [SEQ. ID. NO.: 56] and -1138 to -877 [SEQ. ID. NO.: 57] (with respect to the transcription start determined by RNP). The sequence proximal to the 5' end is strongly homologous (91.4% identity) to the U3 region of long terminal repeats of human endogenous retroviruses HERV-K [Ono, M., "Molecular cloning and long terminal repeat sequences of human endogenous retrovirus genes," J. Virol, 58: 937-944 (1986)]. The LTR-like fragment is 222 bp long with an A-rich tail at its 3' end. Most probably, it represents part of SINE (short interspersed repeated sequence) type nonviral retroposon derived from HERV-K [Ono et al., "A novel human nonviral retroposon derived from an endogenous retrovirus," Nucleic Acids Res., 15: 8725-8373 (1987)]. There are no sequences corresponding to regulatory elements in this fragment, since the 3' part of U3, and the entire R and U5 regions of LTR are absent from the Bd3 genomic clone, and the glucocorticoid responsive element as well as the enhancer core sequences are beyond its 5' border.

However, two keratinocyte-dependent enhancers were identified in the sequence downstream from the LTR-like fragment at positions -3010 and -2814. Those elements are involved in transcriptional regulation of the E6-E7 oncogenes of human papillomaviruses and are thought to account for their tissue specificity [Cripe et al., "Transcriptional regulation of the human papilloma-virus-16 E6-E7 promoter by a keratinocyte-dependent enhancer, and by viral E2 trans-activator and repressor gene products: implications for cervical carcinogenesis," EMBO J., 6: 3745-3753 (1987)].

Nucleotide sequence analysis of the DNA 5' to the transcription start (from nt -507) revealed no recognizable TATA box within the expected distance from the beginning of the first exon (Figure 6). However, the presence of potential

binding sites for transcription factors suggests that this region might contain a promoter for the MN gene. There are several consensus sequences for other regulatory elements, including a p53

5   AP2 as well as for other regulatory elements, including a p53 binding site [Locker and Buzard, "A dictionary of transcription control sequences," _J. DNA Sequencing and Mapping, 1:_   3-11 (1990); Imagawa et al., "Transcription factor AP-2 mediates induction by two different signal-

10   transduction pathways: protein kinase C and cAMP," _Cell, 51:_ 251-260 (1987); El Deiry et al., "Human genomic DNA sequences define a consensus binding site for p53," _Nat. Genet., 1:_   44- 49 (1992)].   Although the putative promoter region contains 59.3% C+G, it does not have additional attributes of CpG-rich

15   islands that are typical for TATA-less promoters of CpG-rich housekeeping genes [Bird, "CpG-rich islands and the function of DNA methylation," _Nature, 321:_ 209-213 (1986)]. Another class of genes lacking TATA box utilizes the initiator (Inr) element as a promoter. Many of these genes are not

20   constitutively active, but they are rather regulated during differentiation or development. The Inr has a consensus sequence of PyPyPyCAPyPyPyPyPy [SEQ. ID. NO.: 23] and encompasses the transcription start site [Smale and Baltimore, "The 'initiator' as a transcription control element," _Cell,_

25   _57:_   103-113 (1989)]. There are two such consensus sequences in the MN putative promoter; however, they do not overlap the transcription start (Figure 6).

           In the initial experiments, the inventors were unable to show promoter activity in human carcinoma cells HeLa

30   and CGL3 that express MN, using the 3.5 kb Bd3 fragment and series of its deletion mutants (from nt -933 to -30) [SEQ. ID. NO.:   58] fused to chloramphenicol acetyl transferase (CAT) gene in a transient system. This might indicate that either the promoter activity of the region 5' to the MN transcription

35   start is below the sensitivity of the CAT assay, or additional regulatory elements not present in our constructs are required for driving the expression of MN gene.

           With respect to this fact, an interesting region was found in the middle of the MN gene. The region is about 1.4

kb in length [nt 4,600-6,000 of the genomic sequence; SEQ. ID.
NO.: 49] and spans from the 3' part of the 1st intron to the
end of the 5th exon. The region has the character of a
typical CpG-rich island, with 62.8% C+G content and 82 CpG:
5   131 GpC dinucleotides. Moreover, there are multiple putative
binding sites for transcription factors AP2 and Sp1 [Locker
and Buzard, supra; Briggs et al., "Purification and
biochemical characterization of the promoter-specific
transcription factor Sp-1," Science, 234: 47-52 (1986)]
10   concentrated in the center of this area. Particularly the 3rd
intron of 131 bp in length contains three Sp1 and three AP2
consensus sequences. That data indicates the possible
involvement of that region in the regulation of MN gene
expression. However, functionality of that region, as well as
15   other regulatory elements found in the proposed 5' MN
promoter, remains to be determined.

MN Promoter Analysis

       To define sequences necessary for MN gene
expression, a series of 5' deletion mutants of the putative
20   promoter region were fused to the bacterial chloramphenicol
acetyltransferase (CAT) gene. [See Figure 8.] The pMN-CAT
deletion constructs were transfected using a DEAE dextran
method for transient expression into HeLa and CGL3 cells.
Those cells were used since they naturally express MN protein,
25   and thus, should contain all the required transcription
factors.
       After 48 hours, crude cell lysates were prepared and
the activity of the expressed CAT was evaluated according to
acetylation of [^{14}C]chloramphenicol by thin layer
30   chromatography. However, no MN promoter CAT activity was
detected in either the HeLa or the CGL3 cells in a transient
system. On the other hand, reporter CAT plasmids with viral
promoters (e.g. pBLV-LTR + tax transactivator, pRSV CAT and
pSV2 CAT), that served as positive controls, gave strong
35   signals on the chromatogram. [pSV2 CAT carries the SV40
origin and expresses CAT from the SV40 early promoter (P_E).

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pRSV CAT expresses CAT from the Rous sarcoma virus (RSV)LTR promoter (P_{LTR}).]

No detectable CAT activity was observed in additional experiments using increasing amounts of transfected plasmids (from 2 to 20 g DNA per dish) and prolonged periods of cell incubation after transcription. Increased cell density also did not improve the results (in contrast to the expectations based on density-dependent expression of native MN protein in HeLa cells). Since the inventors had found consensus sequences for transcription factors AP2 and AP1 in the putative MN promoter, they studied the effect of their inducers dexamethasone (1 m) and phorbol ester phorbol 12-myristate 13-acetate (PMA 50 ng/ml) on CAT activity. However, the MN promoter was unresponsive to those compounds.

The following provides explanations for the results:
--the putative MN promoter immediately preceding the transcription initiation site is very weak, and its activity is below the sensitivity of a standard CAT assay; --additional sequences (e.g enhancers) are necessary for MN transcription.

To further shed light on the regulation of MN expression at the level of transcription, constructs, analogously prepared to the MN-CAT constructs, are prepared, wherein the MN promoter region is upstream from the neomycin phosphotransferase gene engineered for mammalian expression. Such constructs are then transfected to cells which are subjected to selection with G418. Activity of the promoter is then evaluated on the basis of the number of G418 resistant colonies that result. That method has the capacity to detect activity of a promoter that is 50 to 100 times weaker in comparison to promoters detectable by a CAT assay.

Deduced Amino Acid Sequence

The ORF of the MN cDNA shown in Figure 1 has the coding capacity for a 459 amino acid protein with a calculated molecular weight of 49.7 kd. MN protein has an estimated pI of about 4. As assessed by amino acid sequence analysis, the deduced primary structure of the MN protein can be divided into four distinct regions. The initial hydrophobic region of

37 amino acids (AA) corresponds to a signal peptide. The
mature protein has an N-terminal part of 377 AA, a hydrophobic
transmembrane segment of 20 AA and a C-terminal region of 25
AA. Alternatively, the MN protein can be viewed as having
5   five domains as follows: (1) a signal peptide [amino acids
(AA) 1-37; SEQ. ID. NO.: 6]; (2) a region of homology to
collagen alpha1 chain (AA 38-135; SEQ. ID. NO.: 50); (3) a
carbonic anhydrase domain (AA 136-391; SEQ. ID. NO.: 51); (4)
a transmembrane region (AA 414-433; SEQ. ID. NO.: 52); and
10 (5) an intracellular C terminus (AA 434-459; SEQ. ID. NO.:
53). (The AA·numbers are keyed to Figure 1.)

More detailed insight into MN protein primary
structure disclosed the presence of several consensus
sequences. One potential N-glycosylation site was found at
15 position 346 of Figure 1. That feature, together with a
predicted membrane-spanning region are consistent with the
results, in which MN was shown to be an N-glycosylated protein
localized in the plasma membrane. MN protein sequence deduced
from cDNA was also found to contain seven S/TPXX sequence
20 elements [SEQ. ID. NOS.: 25 AND 26] (one of them is in the
signal peptide) defined by Suzuki, J. Mol. Biol., 207: 61-84
(1989) as motifs frequently found in gene regulatory proteins.
However, only two of them are composed of the suggested
consensus amino acids.

25   Experiments have shown that the MN protein is able
to bind zinc cations, as shown by affinity chromatography
using Zn-charged chelating sepharose. MN protein
immunoprecipitated from HeLa cells by Mab M75 was found to
have weak catalytic activity of CA. The CA-like domain of MN
30 has a structural predisposition to serve as a binding site for
small soluble domains. Thus, MN protein could mediate some
kind of signal transduction.

MN protein from LCMV-infected HeLA cells was shown
by using DNA cellulose affinity chromatography to bind to
35 immobilized double-stranded salmon sperm DNA. The binding
activity required both the presence of zinc cations and the
absence of a reducing agent in the binding buffer.

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Sequence Similarities

Computer analysis of the MN cDNA sequence was carried out using DNASIS and PROSID (Pharmacia Software packages). GenBank, EMBL, Protein Identification Resource and
5 SWISS-PROT databases were searched for all possible sequence similarities. In addition, a search for proteins sharing sequence similarities with MN was performed in the MIPS databank with the FastA program [Pearson and Lipman, _PNAS_ (USA), __85__: 2444 (1988)].

10   The MN gene was found to clearly be a novel sequence derived from the human genome. Searches for amino acid sequence similarities in protein databases revealed as the closest homology a level of sequence identity (38.9% in 256 AA or 44% in an 170 AA overlap) between the central part of the
15 MN protein [AAs 136-391 (SEQ. ID. NO: 51)] or 221-390 [SEQ. ID. NO.: 54] of Figure 1 and carbonic anhydrases (CA). However, the overall sequence homology between the cDNA MN sequence and cDNA sequences encoding different CA isoenzymes is in a homology range of 48-50% which is considered by ones
20 in the art to be low. Therefore, the MN cDNA sequence is not closely related to any CA cDNA sequences.

Only very closely related nt sequences having a homology of at least 80-90% would hybridize to each other under stringent conditions. A sequence comparison of the MN
25 cDNA sequence shown in Figure 1 and a corresponding cDNA of the human carbonic anhydrase II (CA II) showed that there are no stretches of identity between the two sequences that would be long enough to allow for a segment of the CA II cDNA sequence having 50 or more nucleotides to hybridize under
30 stringent hybridization conditions to the MN cDNA or vice versa.

Although MN deduced amino acid sequences show some homology to known carbonic anhydrases, they differ from them in several repects. Seven carbonic anhydrases are known
35 [Dodgson et al. (eds.), _The Carbonic Anhydrases_, (Plenum Press; New York/London (1991)]. All of the known carbonic anhydrases are proteins of about 30 kd, smaller than the p54/58N-related products of the MN gene. Further, the

carbonic anhydrases do not form oligomers as do the MN-related
proteins.

The N-terminal part of the MN protein (AA 38-135;
SEQ. ID. NO.: 50) shows a 27-30% identity with human collagen
5   alpha1 chain, which is an important component of the
extracellular matrix.

MN Proteins and/or Polypeptides

The phrase "MN proteins and/or polypeptides" (MN
proteins/polypeptides) is herein defined to mean proteins
10   and/or polypeptides encoded by an MN gene or fragments
thereof. An exemplary and preferred MN protein according to
this invention has the deduced amino acid sequence shown in
Figure 1. Preferred MN proteins/polypeptides are those
proteins and/or polypeptides that have substantial homology
15   with the MN protein shown in Figure 1. For example, such
substantially homologous MN proteins/ polypeptides are those
that are reactive with the MN-specific antibodies of this
invention, preferably the Mabs M75, MN12, MN9 and MN7 or their
equivalents.

20   A "polypeptide" is a chain of amino acids covalently
bound by peptide linkages and is herein considered to be
composed of 50 or less amino acids. A "protein" is herein
defined to be a polypeptide composed of more than 50 amino
acids.

25   MN proteins exhibit several interesting features:
cell membrane localization, cell density dependent expression
in HeLa cells, correlation with the tumorigenic phenotype of
HeLa x fibroblast somatic cell hybrids, and expression in
several human carcinomas among other tissues. As demonstrated
30   herein, for example, in Example 1, MN protein can be found
directly in tumor tissue sections but not in general in
counterpart normal tissues (exceptions noted _infra_ in Example
1 as in normal stomach tissues). MN is also expressed
sometimes in morphologically normal appearing areas of tissue
35   specimens exhibiting dysplasia and/or malignancy. Taken
together, these features suggest a possible involvement of MN

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in the regulation of cell proliferation, differentiation and/or transformation.

It can be appreciated that a protein or polypeptide produced by a neoplastic cell _in vivo_ could be altered in sequence from that produced by a tumor cell in cell culture or
5 by a transformed cell. Thus, MN proteins and/or polypeptides which have varying amino acid sequences including without limitation, amino acid substitutions, extensions, deletions, truncations and combinations thereof, fall within the scope of this invention. It can also be appreciated that a protein
10 extant within body fluids is subject to degradative processes, such as, proteolytic processes; thus, MN proteins that are significantly truncated and MN polypeptides may be found in body fluids, such as, sera. The phrase "MN antigen" is used herein to encompass MN proteins and/or polypeptides.
15 It will further be appreciated that the amino acid sequence of MN proteins and polypeptides can be modified by genetic techniques. Such amino acid changes can be deleted or substituted. One or more amino acids can be deleted or measurable change in the biological activity of the protein or
20 polypeptide and result in proteins or polypeptides which are within the scope of this invention, as well as, MN muteins.

The MN proteins and polypeptides of this invention can be prepared in a variety of ways according to this invention, for example, recombinantly, synthetically or
25 otherwise biologically and/or chemically. A preferred method to prepare MN proteins is by a recombinant means. Particularly preferred methods of recombinantly producing MN proteins are described below for the GEX-3X-MN, MN 20-19, MN-Fc and MN-PA proteins.

30 <u>Recombinant Production of MN Proteins and Polypeptides</u>

A representative method to prepare the MN proteins shown in Figure 1 or fragments thereof would be to insert the full-length or an appropriate fragment of MN cDNA into an
35 appropriate expression vector as exemplified below. In Zavada et al., WO 93/18152, <u>supra</u>, production of a fusion protein

GEX-3X-MN using the partial cDNA clone (described above) in
the vector pGEX-3X (Pharmacia) is described. Nonglycosylated
GEX-3X-MN (the Mn fusion protein MN glutathione S-transferase)
from XL1-Blue cells. Herein described is the recombinant
5 production of both a glycosylated MN protein expressed from
insect cells and a nonglycosylated MN protein expressed from
E. coli using the expression plasmid pEt-22b [Novagen Inc.;
Madison, WI (USA)].

Baculovirus Expression Systems. Recombinant
10 baculovirus express vectors have been developed for infection
into several types of insect cells. For example, recombinant
baculoviruses have been developed for among others: Aedes
aegypti, Autographa californica, Bombyx mor, Drosphila
melanogaster, Heliothis zea, Spodoptera frugiperda, and
15 Trichoplusia ni [PCT Pub. No. WO 89/046699; Wright, Nature,
321: 718 (1986); Fraser et al., In Vitro Cell Dev. Biol., 25:
225 (1989). Methods of introducing exogenous DNA into insect
hosts are well-known in the art. DNA transfection and viral
infection procedures usually vary with the insect genus to be
20 transformed. See, for example, Autographa [Carstens et al.,
Virology, 101: 311 (1980)]; Spodoptera [Kang, "Baculovirus
Vectors for Expression of Foreign Genes," in: Advances in
Virus Research, 35 (1988)]; and Heliothis (virescens) [PCT
Pub. No. WO 88/02030].
25   A wide variety of other host-cloning vector
combinations may be usefully employed in cloning the MN DNA
isolated as described herein. For example, useful cloning
vehicles may include chromosomal, nonchromosomal and synthetic
DNA sequences such as various known bacterial plasmids such as
30 pBR322, other E. coli plasmids and their derivatives and wider
host range plasmids such as RP4, phage DNA, such as, the
numerous derivatives of phage lambda, e.g., NB989 and vectors
derived from combinations of plasmids and phage DNAs such as
plasmids which have been modified to employ phage DNA
35 expression control sequences.
        Useful hosts may be eukaryotic or prokaryotic and
include bacterial hosts such as E. coli and other bacterial
strains, yeasts and other fungi, animal or plant hosts such as

animal or plant cells in culture, insect cells and other
hosts. Of course, not all hosts may be equally efficient.
The particular selection of host-cloning vehicle combination
may be made by those of skill in the art after due
5   consideration of the principles set forth herein without
departing from the scope of this invention.

The particular site chosen for insertion of the
selected DNA fragment into the cloning vehicle to form a
recombinant DNA molecule is determined by a variety of
10   factors. These include size and structure of the protein or
polypeptide to be expressed, susceptibility of the desired
protein or polypeptide to endoenzymatic degradation by the
host cell components and contamination by its proteins,
expression characteristics such as the location of start and
15   stop codons, and other factors recognized by those of skill in
the art.

The recombinant nucleic acid molecule containing the
MN gene, fragment thereof, or cDNA therefrom, may be employed
to transform a host so as to permit that host (transformant)
20   to express the structural gene or fragment thereof and to
produce the protein or polypeptide for which the hybrid DNA
encodes. The recombinant nucleic acid molecule may also be
employed to transform a host so as to permit that host on
replication to produce additional recombinant nucleic acid
25   molecules as a source of MN nucleic acid and fragments
thereof. The selection of an appropriate host for either of
those uses is controlled by a number of factors recognized in
the art. These include, for example, compatibility with the
chosen vector, toxicity of the co-products, ease of recovery
30   of the desired protein or polypeptide, expression
characteristics, biosafety and costs.

Where the host cell is a procaryote such as E. coli,
competent cells which are capable of DNA uptake are prepared
from cells harvested after exponential growth phase and
35   subsequently treated by the $CaCl_2$ method by well known
procedures. Transformation can also be performed after
forming a protoplast of the host cell.

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Where the host used is an eucaryote, transfection methods such as the use of a calcium phosphate-precipitate, electroporation, conventional mechanical procedures such as microinjection, insertion of a plasmid encapsulated in red
5   blood cell ghosts or in liposomes, treatment of cells with agents such as lysophosphatidyl-choline or use of virus vectors, or the like may be used.

The level of production of a protein or polypeptide is governed by three major factors: (1) the number of copies
10 of the gene or DNA sequence encoding for it within the cell; (2) the efficiency with which those gene and sequence copies are transcribed and translated; and (3) the stability of the mRNA. Efficiencies of transcription and translation (which together comprise expression) are in turn dependent upon
15 nucleotide sequences, normally situated ahead of the desired coding sequence. Those nucleotide sequences or expression control sequences define, inter alia, the location at which an RNA polymerase interacts to initiate transcription (the promoter sequence) and at which ribosomes bind and interact
20 with the mRNA (the product of transcription) to initiate translation. Not all such expression control sequences function with equal efficiency. It is thus of advantage to separate the specific coding sequences for the desired protein from their adjacent nucleotide sequences and fuse them instead
25 to known expression control sequences so as to favor higher levels of expression. This having been achieved, the newly engineered DNA fragment may be inserted into a multicopy plasmid or a bacteriophage derivative in order to increase the number of gene or sequence copies within the cell and thereby
30 further improve the yield of expressed protein.

Several expression control sequences may be employed. These include the operator, promoter and ribosome binding and interaction sequences (including sequences such as the Shine-Dalgarno sequences) of the lactose operon of E. coli
35 ("the lac system"), the corresponding sequences of E. coli tryptophan synthetase system of E. coli ("the trp system"), the fusion of the trp and lac promoter ("the tac system"), a major operator and promoter regions of phage lambda ($O_L P_L$ and

O_RP_R,), and the control region of the phage fd coat protein.
DNA fragments containing these sequences are excised by
cleavage with restriction enzymes from the DNA isolated from
transducing phages that carry the lac or trp operons, or from

5   the DNA of phage lambda or fd. Those fragments are then .
manipulated in order to obtain a limited population of
molecules such that the essential controlling sequences can be
joined very close to, or in juxtaposition with, the initiation
codon of the coding sequence.

10   The fusion product is then inserted into a cloning
vehicle for transformation or transfection of the appropriate
hosts and the level of antigen production is measured. Cells
giving the most efficient expression may be thus selected.
Alternatively, cloning vechicles carrying the lac, trp or

15   lambda P_L control system attached to an initiation codon may be
employed and fused to a fragment containing a sequence coding
for a MN protein or polypeptide such that the gene or sequence
is correctly translated from the initiation codon of the
cloning vehicle.

20   The phrase "recombinant nucleic acid molecule" is
herein defined to mean a hybrid nucleotide sequence comprising
at least two nucleotide sequences, the first sequence not
normally being found together in nature with the second.
       The phrase "expression control sequence" is herein

25   defined to mean a sequence of nucleotides that controls and
regulates expression of structural genes when operatively
linked to those genes.
       The following are representative examples of
genetically engineering MN proteins of this invention. The

30   descriptions are exemplary and not meant to limit the
invention in any way.

Expression of MN 20-19 Protein
       A representative, recombinantly produced MN protein
of this invention is the MN 20-19 protein which, when produced

35   in baculovirus-infected Sf9 cells [Spodoptera frugiperda
cells; Clontech; Palo Alto, CA (USA)], is glycosylated. The
MN 20-19 protein misses the putative signal peptide (AAs 1-37)

of SEQ. ID. NO.: 6 (Figure 1), has a methionine (Met) at the
N-terminus for expression, and a Leu-Glu-His-His-His-His-His-
His [SEQ. ID NO.: 22] added to the C-terminus for
purification.

5   In order to insert the portion of the MN coding
sequence for the GEX-3X-MN fusion protein into alternate
expression systems, a set of primers for PCR was designed.
The primers were constructed to provide restriction sites at
each end of the coding sequence, as well as in-frame start and
10   stop codons. The sequences of the primers, indicating
restriction enzyme cleavage sites and expression landmarks,
are shown below.

Primer #20:N-terminus

                              ┌Translation start
15   5'GTC**GCTAGC**T**CCATGGG**T**CATATG**CAGAGGTTGCCCCGGATGCAG 3'
          NheI   NcoI   NdeI └MN cDNA #1   [SEQ. ID. NO. 17]

Primer #19:C-terminus

                    ┌Translation stop
     5'GA**AGATCT**CTTA**CTCGAG**CATTCTCCAAGATCCAGCCTCTAGG 3'
20   BglII   XhoI └MN cDNA   [SEQ. ID. NO. 18]

The SEQ. ID. NOS.: 17 and 18 primers were used to amplify the
MN coding sequence present in the GEX-3X-MN vector using
standard PCR techniques. The resulting PCR product (termed MN
20-19) was electrophoresed on a 0.5% agarose/1X TBE gel; the
25   1.3 kb band was excised; and the DNA recovered using the Gene
Clean II kit according to the manufacturer's instructions
[Bio101; LaJolla, CA (USA)].
          MN 20-19 and plasmid pET-22b were cleaved with the
restriction enzymes NdeI and XhoI, phenol-chloroform
30   extracted, and the appropriate bands recovered by agarose gel
electrophoresis as above. The isolated fragments were ethanol
co-precipitated at a vector:insert ratio of 1:4. After

resuspension, the fragments were ligated using T4 DNA ligase.
The resulting product was used to transform competent Novablue
E. coli cells [Novagen, Inc.]. Plasmid mini-preps [Magic
Minipreps; Promega] from the resultant ampicillin resistant

5   colonies were screened for the presence of the correct insert
by restriction mapping. Insertion of the gene fragment into
the pET-22b plasmid using the NdeI and XhoI sites added a 6-
histidine tail to the protein that could be used for affinity
isolation.

10   To prepare MN 20-19 for insertion into the
baculovirus expression system, the MN 20-19 gene fragment was
excised from pET-22b using the restriction endonucleases XbaI
and PvuI. The baculovirus shuttle vector pBacPAK8 [Clontech]
was cleaved with XbaI and PacI. The desired fragments (1.3 kb

15   for MN 20-19 and 5.5 kb for pBacPAK8) were isolated by agarose
gel electrophoresis, recovered using Gene Clean II, and co-
precipitated at an insert:vector ratio of 2.4:1.

          After ligation with T4 DNA ligase, the DNA was used
to transform competent NM522 E. coli cells (Stratagene).

20   Plasmid mini-preps from resultant ampicillin resistant
colonies were screened for the presence of the correct insert
by restriction mapping. Plasmid DNA from an appropriate
colony and linearized BacPAK6 baculovirus DNA [Clontech] were
used to transform Sf9 cells by standard techniques.

25   Recombination produced BacPAK viruses carrying the MN 20-19
sequence. Those viruses were plated onto Sf9 cells and
overlaid with agar.

          Plaques were picked and plated onto Sf9 cells. The
conditioned media and cells were collected. A small aliquot

30   of the conditioned media was set aside for testing. The cells
were extracted with PBS with 1% Triton X100.

          The conditioned media and the cell extracts were dot
blotted onto nitrocellulose paper. The blot was blocked with
5% non-fat dried milk in PBS. Mab M75 were used to detect the

35   MN 20-19 protein in the dot blots. A rabbit anti-mouse Ig-HRP
was used to detect bound Mab M75. The blots were developed
with TMB/H_2O_2 with a membrane enhancer [KPL; Gaithersburg, MD
(USA)]. Two clones producing the strongest reaction on the

dot blots were selected for expansion. One was used to produce MN 20-19 protein in High Five cells [Invitrogen Corp., San Diego, CA (USA); BTI-TN-5BI-4; derived from _Trichoplusia ni_ egg cell homogenate]. MN 20-19 protein was purified from
5   the conditioned media from the virus infected High Five cells.

The MN 20-19 protein was purified from the conditioned media by immunoaffinity chromatography. 6.5 mg of Mab M75 was coupled to 1 g of Tresyl activated Toyopearl™ [Tosoh, Japan (#14471)]. Approximately 150 ml of the
10   conditioned media was run through the M75-Toyopearl column. The column was washed with PBS, and the MN 20-19 protein was eluted with 1.5 M MgCl. The eluted protein was then dialyzed against PBS.

Fusion Proteins with C-Terminal Part Including Transmembrane
15   Region Replaced by Fc or PA

MN fusion proteins in which the C terminal part including the transmembrane region is replaced by the Fc fragment of human IgG or by Protein A were constructed. Such fusion proteins are useful to identify MN binding protein(s).
20   In such MN chimaeras, the whole N-terminal part of MN is accessible to interaction with heterologous proteins, and the C terminal tag serves for simple detection and purification of protein complexes.

Fusion Protein MN-PA (Protein A)
25   In a first step, the 3' end of the MN cDNA encoding the transmembrane region of the MN protein was deleted. The plasmid pFLMN (e.g. pBluescript with full length MN cDNA) was cleaved by _EcoRI_ and blunt ended by S1 nuclease. Subsequent cleavage by _SacI_ resulted in the removal of the _EcoRI-SacI_
30   fragment. The deleted fragment was then replaced by a Protein A coding sequence that was derived from plasmid pEZZ (purchased from Pharmacia), which had been cleaved with _RsaI_ and _SacI_. The obtained MN-PA construct was subcloned into a eukaryotic expression vector pSG5C (described in Example 3),
35   and was then ready for transfection experiments.

Fusion Protein MN-Fc

The cloning of the fusion protein MN-Fc was rather complicated due to the use of a genomic clone containing th Fc fragment of human IgG which had a complex structure in that

5   it contained an enhancer, a promoter, exons and introns. Moreover, the complete sequence of the clone was not available. Thus, it was necessary to ensure the correct in-phase splicing and fusion of MN to the Fc fragment by the addition of a synthetic splice donor site (SSDS) designed

10 according to the splicing sequences of the MN gene.

The construction procedure was as follows:

1. Plasmid pMH4 (e.g. pSV2gpt containing a genomic clone of the human IgG Fc region) was cleaved by BamHI in order to get a 13 kb fragment encoding Fc. [In pSV2gpt, the

15 E. coli xanthine-guanine phosphoribosyl transferase gene (gpt) is expressed using the SV40 early promoter (P_E) located in the SV40 origin, the SV40 small T intron, and the SV40 polyadenylation site.]

2. At the same time, plasmid pFLMN (with full

20 length MN cDNA) was cleaved by SalI-EcoRI. The released fragment was purified and ligated with a synthetic adapter EcoRI-BglII containing a synthetic splice donor site (SSDS).

3. Simultaneously, the plasmid pBKCMV was cleaved by SalI-BamHI. Then advantage was taken of the fact that the

25 BamHI cohesive ends (of the Fc coding fragment) are compatible with the BglII ends of the SSDS, and Fc was ligated to MN. The MN-Fc ligation product was then inserted into pBKCMV by directional cloning through the SalI and BamHI sites.

Verification of the correct orientation and in-phase

30 fusion of the obtained MN-Fc chimaeric clones was problematic in that the sequence of Fc was not known. Thus, functional constructs are selected on the basis of results of transient eukaryotic expression analyses.

Synthetic and Biologic Production of
MN Proteins and Polypeptides

35

MN proteins and polypeptides of this invention may be prepared not only by recombinant means but also by

synthetic and by other biologic means. Synthetic formation of the polypeptide or protein requires chemically synthesizing the desired chain f amino acids by methods well known in the art. Exemplary of other biologic means to prepare the desired

5 polypeptide or protein is to subject to selective proteolysis a longer MN polypeptide or protein containing the desired amino acid sequence; for example, the longer polypeptide or protein can be split with chemical reagents or with enzymes.

Chemical synthesis of a peptide is conventional in

10 the art and can be accomplished, for example, by the Merrifield solid phase synthesis technique [Merrifield, J., Am. Chem. Soc., 85: 2149-2154 (1963); Kent et al., Synthetic Peptides in Biology and Medicine, 29 f.f. eds. Alitalo et al., (Elsevier Science Publishers 1985); and Haug, J.D., "Peptide

15 Synthesis and Protecting Group Strategy", American Biotechnology Laboratory, 5(1): 40-47 (Jan/Feb. 1987)].

Techniques of chemical peptide synthesis include using automatic peptide synthesizers employing commercially available protected amino acids, for example, Biosearch [San

20 Rafael, CA (USA)] Models 9500 and 9600; Applied Biosystems, Inc. [Foster City, CA (USA)] Model 430; Milligen [a division of Millipore Corp.; Bedford, MA (USA)] Model 9050; and Du Pont's RAMP (Rapid Automated Multiple Peptide Synthesis) [Du Pont Compass, Wilmington, DE (USA)].

25   Regulation of MN Expression and MN Promoter
MN appears to be a novel regulatory protein that is directly involved in the control of cell proliferation and in cellular transformation. In HeLa cells, the expression of MN is positively regulated by cell density. Its level is

30 increased by persistent infection with LCMV. In hybrid cells between HeLa and normal fibroblasts, MN expression correlates with tumorigenicity. The fact that MN is not present in nontumorigenic hybrid cells (CGL1), but is expressed in a tumorigenic segregant lacking chromosome 11, indicates that MN

35 is negatively regulated by a putative suppressor in chromosome 11.

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Evidence supporting the regulatory role of MN
protein was found in the generation of stable transfectants of
NIH 3T3 cells that constitutively express MN protein as
described in Example 3. As a consequence of MN expression,
5   the NIH 3T3 cells acquired features associated with a
transformed phenotype: altered morphology, increased
saturation density, proliferative advantage in serum-reduced
media, enhanced DNA synthesis and capacity for anchorage-
independent growth. Further, as shown in Example 4, flow
10   cytometric analyses of asynchronous cell populations indicated
that the expression of MN protein leads to accelerated
progression of cells through G1 phase, reduction of cell size
and the loss of capacity for growth arrest under inappropriate
conditions. Also, Example 4 shows that MN expressing cells
15   display a decreased sensitivity to the DNA damaging drug
mitomycin C.

Nontumorigenic human cells, CGL1 cells, were also
transfected with the full-length MN cDNA. The same pSG5C-MN
construct in combination with pSV2neo plasmid as used to
20   transfect the NIH 3T3 cells (Example 3) was used. Also the
protocol was the same except that the G418 concentration was
increased to 1000 μg/ml.

Out of 15 MN-positive clones (tested by SP-RIA and
Western blotting), 3 were chosen for further analysis. Two
25   MN-negative clones isolated from CGL1 cells transfected with
empty plasmid were added as controls. Initial analysis
indicates that the morphology and growth habits of MN-
transfected CGL1 cells are not changed dramatically, but their
proliferation rate and plating efficiency is increased.

30   MN cDNA and promoter. When the promoter region from
the MN genomic clone, isolated as described above, was linked
to MN cDNA and transfected into CGL1 hybrid cells, expression
of MN protein was detectable immediately after selection.
However, then it gradually ceased, indicating thus an action
35   of a feedback regulator. The putative regulatory element
appeared to be acting via the MN promoter, because when the
full-length cDNA (not containing the promoter) was used for
transfection, no similar effect was observed.

An "antisense" MN cDNA/MN promoter construct was
used to transfect CGL3 cells. The effect was the opposite of
that of the CGL1 cells transfected with the "sense" construct.
Whereas the transfected CGL1 cells formed colonies several
5   times larger than the control CGL1, the transfected CGL3 cells
formed colonies much smaller than the control CGL3 cells.

For those experiments, the part of the promoter
region that was linked to the MN cDNA through a BamHI site was
derived from a NcoI - BamHI fragment of the MN genomic clone
10   [Bd3] and represents a region a few hundred bp upstream from
the transcription initiation site. After the ligation, the
joint DNA was inserted into a pBK-CMV expression vector
[Stratagene]. The required orientation of the inserted
sequence was ensured by directional cloning and subsequently
15   verified by restriction analysis. The tranfection procedure
was the same as used in transfecting the NIH 3T3 cells
(Example 3), but co-transfection with the pSV2neo plasmid was
not necessary since the neo selection marker was already
included in the pBK-CMV vector.

20   After two weeks of selection in a medium containing
G418, remarkable differences between the numbers and sizes of
the colonies grown were evident as noted above. Immediately
following the selection and cloning, the MN-transfected CGL1
and CGL3 cells were tested by SP-RIA for expression and
25   repression of MN, respectively. The isolated transfected CGL1
clones were MN positive (although the level was lower than
obtained with the full-length cDNA), whereas MN protein was
almost absent from the transfected CGL3 clones. However, in
subsequent passages, the expression of MN in transfected CGL1
30   cells started to cease, and was then blocked perhaps
evidencing a control feedback mechanism.

As a result of the very much lowered proliferation
of the transfected CGL3 cells, it was difficult to expand the
majority of cloned cells (according to SP-RIA, those with the
35   lowest levels of MN), and they were lost during passaging.
However, some clones overcame that problem and again expressed
MN. It is possible that once those cells reached a higher
quantity, that the level of endogenously produced MN mRNA

increased over the amount of ectopically expressed antisense
mRNA.

Transformation and Reversion

As illustrated in Examples 3 and 4, vertebrate cells
5   transfected with MN cDNA in suitable vectors show striking
morphologic transformation. Transformed cells may be very
small, densely packed, slowly growing, with basophilic
cytoplasm and enlarged Golgi apparatus. However, it has been
found that transformed clones revert over time, for example,
10   within 3-4 weeks, to nearly normal morphology, even though the
cells may be producing MN protein at high levels. MN protein
is biologically active even in yeast cells; depending upon the
level of its expression, it stimulates or retards their growth
and induces morphologic alterations.
15   Full-length MN cDNA was inserted into pGD, a MLV-
derived vector, which together with standard competent MLV
(murine leukemia virus), forms an infectious, transmissible
complex [pGD-MN + MLV]. That complex also transforms
vertebrate cells, such as, NIH 3T3 cells and mouse embryo
20   fibroblasts BALB/c, which also revert to nearly normal
morphology. Such revertants again contain MN protein and
produce the [pGD-MN + MLV] artificial virus complex, which
retains its transforming capacity. Thus, reversion of MN-
transformed cells is apparently not due to a loss, silencing
25   or mutation of MN cDNA, but may be the result of the
activation of suppressor gene(s).

Nucleic Acid Probes and Test Kits

Nucleic acid probes of this invention are those
comprising sequences that are complementary or substantially
30   complementary to the MN cDNA sequence shown in Figure 1 or to
other MN gene sequences, such as, the complete genomic
sequence of Figure 3a-d [SEQ. ID. NO.: 5] and the putative
promoter sequence [SEQ. ID. NO.: 27 of Figure 6]. The phrase
"substantially complementary" is defined herein to have the
35   meaning as it is well understood in the art and, thus, used in
the context of standard hybridization conditions. The

stringency of hybridization conditions can be adjusted to control the precision of complementarity. Two nucleic acids are, for example, substantially complementary to each other, if they hybridize to each other under stringent hybridization

5   conditions.

Stringent hybridization conditions are considered herein to conform to standard hybridization conditions :
understood in the art to be stringent. For example, it is generally understood that stringent conditions encompass

10   relatively low salt and/or high temperature conditions, such as provided by 0.02 M to 0.15 M NaCl at temperatures of 50°C to 70°C. Less stringent conditions, such as, 0.15 M to 0.9 M salt at temperatures ranging from 20°C to 55°C can be made more stringent by adding increasing amounts of formamide, which

15   serves to destabilize hybrid duplexes as does increased temperature.

Exemplary stringent hybridization conditions are described in Sambrook et al., Molecular Cloning: A Laboratory Manual, pages 1.91 and 9.47-9.51 (Second Edition, Cold Spring

20   Harbor Laboratory Press; Cold Spring Harbor, NY; 1989); Maniatis et al., Molecular Cloning: A Laboratory Manual, pages 387-389 (Cold Spring Harbor Laboratory; Cold Spring Harbor, NY; 1982); Tsuchiya et al., Oral Surgery, Oral Medicine, Oral Pathology, 71(6): 721-725 (June 1991).

25   Preferred nucleic acid probes of this invention are fragments of the isolated nucleic acid sequences that encode MN proteins or polypeptides according to this invention. Preferably those probes are composed of at least twenty-nine nucleotides, more preferably, fifty nucleotides.

30   Nucleic acid probes of this invention need not hybridize to a coding region of MN. For example, nucleic acid probes of this invention may hybridize partially or wholly to a non-coding region of the genomic sequence shown in Figure 3a-d [SEQ. ID. NO.: 5]. Conventional technology can be used

35   to determine whether fragments of SEQ. ID. NO.: 5 or related nucleic acids are useful to identify MN nucleic acid sequences. [See, for example, Benton and Davis, supra and Fuscoe et al., supra.]

Areas of homology of the MN nt sequence to other
non-MN nt sequences are indicated above. In general,
nucleotide sequences that are not in the Alu or LTR-like
regions, of preferably 29 bases or more, or still more
5 preferably of 50 bases or more, can be routinely tested and
screened and found to hybridize under stringent conditions to
only MN nucleotide sequences. Further, not all homologies
within the Alu-like MN genomic sequences are so close to Alu
repeats as to give a hybridization signal under stringent
10 hybridization conditions. The percent of homology between MN
Alu-like regions and a standard Alu-J sequence are indicated
as follows:

Region of Homology within MN Genomic Sequence [SEQ. ID. NO.: 5; Figure 3a-d]	SEQ. ID. NOS.	% Homology to Entire Alu-J Sequence
921-1212	59	89.1%
2370-2631	60	78.6%
4587-4880	61	90.1%
6463-6738	62	85.4%
7651-7939	63	91.0%
9020-9317	64	69.8%

		% Homology to One Half of Alu-J Sequence
8301-8405	65	88.8%
10040-10122	66	73.2%.

25   Nucleic acid probes of this invention can be used to
detect MN DNA and/or RNA, and thus can be used to test for the
presence or absence of MN genes, and amplification(s),
mutation(s) or genetic rearrangements of MN genes in the cells
of a patient. For example, overexpression of an MN gene may
30 be detected by Northern blotting and RNase protection analysis
using probes of this invention. Gene alterations, as
amplifications, translocations, inversions, and deletions
among others, can be detected by using probes of this

invention for _in situ_ hybridization to chromosomes from a
patient's cells, whether in metaphase spreads or interphase
nuclei.   Southern blotting could also be used with the probes
of this invention to detect amplifications or deletions of MN
5   genes.   Restriction Fragment Length Polymorphism (RFLP)
analysis using said probes is a preferred method of detecting
gene alterations, mutations and deletions.   Said probes can
also be used to identify MN proteins and/or polypeptides as
well as homologs or near homologs thereto by their
10   hybridization to various mRNAs transcribed from MN genes in
different tissues.

            Probes of this invention thus can be useful
diagnostically/prognostically.   Said probes can be embodied in
test kits, preferably with appropriate means to enable said
15   probes when hybridized to an appropriate MN gene or MN mRNA
target to be visualized.   Such samples include tissue
specimens including smears, body fluids and tissue and cell
extracts.

                          PCR Assays
20   To detect relatively large genetic rearrangements,
hybridization tests can be used.   To detect relatively small
genetic rearrangements, as, for example, small deletions or
amplifications, or point mutations, PCR would preferably be
used.   [U.S. Patent Nos. 4,800,159; 4,683,195; 4,683,202; and
25   Chapter 14 of Sambrook et al., _Molecular Cloning: A_
Laboratory Manual, supra]

            An exemplary assay would use cellular DNA from
normal and cancerous cells, which DNA would be isolated and
amplified employing appropriate PCR primers.   The PCR products
30   would be compared, preferably initially, on a sizing gel to
detect size changes indicative of certain genetic
rearrangements.   If no differences in sizes are noted, further
comparisons can be made, preferably using, for example, PCR-
single-strand conformation polymorphism (PCR-SSCP) assay or a
35   denaturing gradient gel electrophoretic assay.   [_See_, for
ex..rple, Hayashi, K., "PCR-SSCP: A Simple and Sensitive
Method for Detection of Mutations in the Genomic DNA," in _PCR_

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Methods and Applications, 1: 34-38 (1991); and Meyers et al.,
"Detection and Localization of Single Base Changes by
Denaturing Gradient Gel Electrophoresis," Methods in
Enzymology, 155: 501 (1987).]

5   Assays
          Assays according to this invention are provided to
detect and/or quantitate MN antigen or MN-specific antibodies
in vertebrate samples, preferably mammalian samples, more
preferably human samples. Such samples include tissue
10 specimens, body fluids, tissue extracts and cell extracts. MN
antigen may be detected by immunoassay, immunohistochemical
staining, immunoelectron and scanning microscopy using
immunogold among other techniques.
          Preferred tissue specimens to assay by
15 immunohistochemical staining include cell smears, histological
sections from biopsied tissues or organs, and imprint
preparations among other tissue samples. Such tissue
specimens can be variously maintained, for example, they can
be fresh, frozen, or formalin-, alcohol- or acetone- or
20 otherwise fixed and/or paraffin-embedded and deparaffinized.
Biopsied tissue samples can be, for example, those samples
removed by aspiration, bite, brush, cone, chorionic villus,
endoscopic, excisional, incisional, needle, percutaneous
punch, and surface biopsies, among other biopsy techniques.
25   Preferred cervical tissue specimens include cervical
smears, conization specimens, histologic sections from
hysterectomy specimens or other biopsied cervical tissue
samples. Preferred means of obtaining cervical smears include
routine swab, scraping or cytobrush techniques, among other
30 means. More preferred are cytobrush or swab techniques.
Preferably, cell smears are made on microscope slides, fixed,
for example, with 55% EtOH or an alcohol based spray fixative
and air-dried.
          Papanicolaou-stained cervical smears (Pap smears)
35 can be screened by the methods of this invention, for example,
for retrospective studies. Preferably, Pap smears would be
decolorized and re-stained with labeled antibodies against MN

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antigen. Also archival specimens, for example, matched smears and biopsy and/or tumor specimens, can be used for retrospective studies. Prospective studies can also be done with matched specimens from patients that have a higher than

5 normal risk of exhibiting abnormal cervical cytopathology.

Preferred samples in which to assay MN antigen by, for example, Western blotting or radioimmunoassay, are tissue and/or cell extracts. However, MN antigen may be detected in body fluids, which can include among other fluids: blood,

10 serum, plasma, semen, breast exudate, saliva, tears, sputum, mucous, urine, lymph, cytosols, ascites, pleural effusions, amniotic fluid, bladder washes, bronchioalveolar lavages and cerebrospinal fluid. It is preferred that the MN antigen be concentrated from a larger volume of body fluid before

15 testing. Preferred body fluids to assay would depend on the type of cancer for which one was testing, but in general preferred body fluids would be breast exudate, pleural effusions and ascites.

MN-specific antibodies can be bound by serologically

20 active MN proteins/polypeptides in samples of such body fluids as blood, plasma, serum, lymph, mucous, tears, urine, spinal fluid and saliva; however, such antibodies are found most usually in blood, plasma and serum, preferably in serum.

Correlation of the results from the assays to detect and/or

25 quantitate MN antigen and MN-specific antibodies reactive therewith, provides a preferred profile of the disease condition of a patient.

The assays of this invention are both diagnostic and/or prognostic, i.e., diagnostic/prognostic. The term

30 "diagnostic/ prognostic" is herein defined to encompass the following processes either individually or cumulatively depending upon the clinical context: determining the presence of disease, determining the nature of a disease, distinguishing one disease from another, forecasting the prospect

35 probable outcome of a disease state, determining the nature and as to recovery from a disease as indicated by the nature and symptoms of a case, monitoring the disease status of a patient, monitoring a patient for recurrence of disease,

and/or determining the preferred therapeutic regimen for a
patient. The diagnostic/prognostic methods of this invention
are useful, for example, for screening populations for the
presence of neoplastic or pre-neoplastic disease, determining
5 the risk of developing neoplastic disease, diagnosing the
presence of neoplastic and/or pre-neoplastic disease,
monitoring the disease status of patients with neoplastic
disease, and/or determining the prognosis for the course of
neoplastic disease. For example, it. appears that the
10 intensity of the immunostaining with MN-specific antibodies
may correlate with the severity of dysplasia present in
samples tested.

The present invention is useful for screening for
the presence of a wide variety of neoplastic diseases as
15 indicated above. The invention provides methods and
compositions for evaluating the probability of the presence of
malignant or pre-malignant cells, for example, in a group of
cells freshly removed from a host. Such an assay can be used
to detect tumors, quantitate their growth, and help in the
20 diagnosis and prognosis of disease. The assays can also be
used to detect the presence of cancer metastasis, as well as
confirm the absence or removal of all tumor tissue following
surgery, cancer chemotherapy and/or radiation therapy. It can
further be used to monitor cancer chemotherapy and tumor
25 reappearance.

The presence of MN antigen or antibodies can be
detected and/or quantitated using a number of well-defined
diagnostic assays. Those in the art can adapt any of the
conventional immunoassay formats to detect and/or quantitate
30 MN antigen and/or antibodies.

Many formats for detection of MN antigen and MN-
specific antibodies are, of course available. Those can be
Western blots, ELISAs, RIAs, competitive EIA or dual antibody
sandwich assays, immunohistochemical staining, among other
35 assays all commonly used in the diagnostic industry. In such
immunoassays, the interpretation of the results is based on
the assumption that the antibody or antibody combination will

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not cross-react with other proteins and protein fragments present in the sample that are unrelated to MN.

Representative of one type of ELISA test for MN antigen is a format wherein a microtiter plate is coated with 5 antibodies made to MN proteins/polypeptides or antibodies made to whole cells expressing MN proteins, and to this is added a patient sample, for example, a tissue or cell extract. After a period of incubation permitting any antigen to bind to the antibodies, the plate is washed and another set of anti-MN 10 antibodies which are linked to an enzyme is added, incubated to allow reaction to take place, and the plate is then rewashed. Thereafter, enzyme substrate is added to the microtiter plate and incubated for a period of time to allow the enzyme to work on the substrate, and the absorbance of the 15 final preparation is measured. A large change in absorbance indicates a positive result.

It is also apparent to one skilled in the art of immunoassays that MN proteins and/or polypeptides can be used to detect and/or quantitate the presence of MN antigen in the 20 body fluids, tissues and/or cells of patients. In one such embodiment, a competition immunoassay is used, wherein the MN protein/polypeptide is labeled and a body fluid is added to compete the binding of the labeled MN protein/polypeptide.

In another embodiment, an immunometric assay may be 25 used wherein a labeled antibody made to a MN protein or polypeptide is used. In such an assay, the amount of labeled antibody which complexes with the antigen-bound antibody is directly proportional to the amount of MN antigen in the sample.

30 A representative assay to detect MN-specific antibodies is a competition assay in which labeled MN protein/polypeptide is precipitated by antibodies in a sample, for example, in combination with monoclonal antibodies recognizing MN proteins/polypeptides. One skilled in the art 35 could adapt any of the conventional immunoassay formats to detect and/or quantitate MN-specific antibodies. Detection of the binding of said antibodies to said MN protein/polypeptide

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could be by many ways known to those in the art, e.g., in
humans with the use of anti-human labeled IgG.

      An exemplary immunoassay method of this invention to
detect and/or quantitate MN antigen in a vertebrate sample
5   comprises the steps of:

      a) incubating said vertebrate sample with one or
more sets of antibodies (an antibody or antibodies) that bind
to MN antigen wherein one set is labeled or otherwise
detectable;

10   b) examining the incubated sample for the presence
of immune complexes comprising MN antigen and said antibodies.

      Another exemplary immunoassay method according to
this invention is that wherein a competition immunoassay is
used to detect and/or quantitate MN antigen in a vertebrate
15   sample and wherein said method comprises the steps of:

      a) incubating a vertebrate sample with one or more
sets of MN-specific antibodies and a certain amount of a
labeled or otherwise detectable MN protein/polypeptide wherein
said MN protein/ polypeptide competes for binding to said
20   antibodies with MN antigen present in the sample;

      b) examining the incubated sample to determine the
amount of labeled/detectable MN protein/polypeptide bound to
said antibodies; and

      c) determining from the results of the examination
25   in step b) whether MN antigen is present in said sample and/or
the amount of MN antigen present in said sample.

      Once antibodies (including biologically active
antibody fragments) having suitable specificity have been
prepared, a wide variety of immunological assay methods are
30   available for determining the formation of specific
antibody-antigen complexes. Numerous competitive and
non-competitive protein binding assays have been described in
the scientific and patent literature, and a large number of
such assays are commercially available. Exemplary
35   immunoassays which are suitable for detecting a serum antigen
include those described in U.S. Patent Nos. 3,984,533;
3,996,345; 4,034,074; and 4,098,876.

Antibodies employed in assays may be labeled or unlabeled. Unlabeled antibodies may be employed in agglutination; labeled antibodies may be employed in a wide variety of assays, employing a wide variety of labels.

Suitable detection means include the use of labels such as radionuclides, enzymes, coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, free radicals, particles, dyes and the like. Such labeled reagents may be used in a variety of well known assays, such as radioimmunoassays, enzyme immunoassays, e.g., ELISA, fluorescent immunoassays, and the like. See for example, U.S. Patent Nos. 3,766,162; 3,791,932; 3,817,837; and 4,233,402.

Immunoassay Test Kits

The above outlined assays can be embodied in test kits to detect and/or quantitate MN antigen and/or MN-specific antibodies (including biologically active antibody fragments). Kits to detect and/or quantitate MN antigen can comprise MN protein(s)/polypeptides(s) and/or MN-specific antibodies, polyclonal and/or monoclonal. Such diagnostic/prognostic test kits can comprise one or more sets of antibodies, polyclonal and/or monoclonal, for a sandwich format wherein antibodies recognize epitopes on the MN antigen, and one set is appropriately labeled or is otherwise detectable.

Test kits for an assay format wherein there is competition between a labeled (or otherwise detectable) MN protein/polypeptide and MN antigen in the sample, for binding to an antibody, can comprise the combination of the labeled protein/polypeptide and the antibody in amounts which provide for optimum sensitivity and accuracy.

Test kits for MN-specific antibodies preferably comprise labeled/detectable MN proteins(s) and/or polypeptides(s), and may comprise other components as necessary, such as, controls, buffers, diluents and detergents. Such test kits can have other appropriate formats for conventional assays.

A kit for use in an enzyme-immunoassay typically
includes an enzyme-labelled reagent and a substrate for the
enzyme. The enzyme can, for example, bind either an MN-
specific antibody of this invention or to an antibody to such
5 an MN-specific antibody.

Preparation of MN-Specific Antibodies

The term "antibodies" is defined herein to include
not only whole antibodies but also biologically active
fragments of antibodies, preferably fragments containing the
10 antigen binding regions. Such antibodies may be prepared by
conventional methodology and/or by genetic engineering.
Antibody fragments may be genetically engineered, preferably
from the variable regions of the light and/or heavy chains (V_H
and V_L), including the hypervariable regions, and still more
15 preferably from both the V_H and V_L regions. For example, the
term "antibodies" as used herein comprehends polyclonal and
monoclonal antibodies and biologically active fragments
thereof including among other possibilities "univalent"
antibodies [Glennie et al., Nature, 295: 712 (1982)]; Fab
20 proteins including Fab' and F(ab')$_2$ fragments whether
covalently or non-covalently aggregated; light or heavy chains
alone, preferably variable heavy and light chain regions (V_H
and V_L regions), and more preferably including the
hypervariable regions [otherwise known as the complementarity
25 determining regions (CDRs) of said V_H and V_L regions]; F_c
proteins; "hybrid" antibodies capable of binding more than one
antigen; constant-variable region chimeras; "composite"
immunoglobulins with heavy and light chains of different
origins; "altered" antibodies with improved specificity and
30 other characteristics as prepared by standard recombinant
techniques and also by oligonucleotide-directed mutagenesis
techniques [Dalbadie-McFarland et al., PNAS (USA), 79: 6409
(1982)].

It may be preferred for therapeutic and/or imaging
35 uses that the antibodies be biologically active antibody
fragments, preferably genetically engineered fragments, more
preferably genetically engineered fragments from the V_H and/or

V_L regions, and still more preferably comprising the hypervariable regions thereof.

There are conventional techniques for making polyclonal and monoclonal antibodies well-known in the

5 immunoassay art.   Immunogens to prepare MN-specific antibodies include MN proteins and/or polypeptides, preferably purified, and MX-infected tumor line cells, for example, MX-infected HeLa cells, among other immunogens.

Anti-peptide antibodies are also made by

10 conventional methods in the art as described in European Patent Publication No. 44,710 (published Jan. 27, 1982). Briefly, such anti-peptide antibodies are prepared by selecting a peptide from an MN amino acid sequence as from Figure 1, chemically synthesizing it, conjugating it to an

15 appropriate immunogenic protein and injecting it into an appropriate animal, usually a rabbit or a mouse; then, either polyclonal or monoclonal antibodies are made, the latter by a Kohler-Milstein procedure, for example.

Besides conventional hybridoma technology, newer

20 technologies can be used to produce antibodies according to this invention. For example, the use of the PCR to clone and express antibody V-genes and phage display technology to select antibody genes encoding fragments with binding activities has resulted in the isolation of antibody fragments

25 from repertoires of PCR amplified V-genes using immunized mice or humans. [Marks et al., BioTechnology, 10: 779 (July 1992) for references; Chiang et al., BioTechniques, 7(4): 360 (1989); Ward et al., Nature, 341: 544 (Oct. 12, 1989); Marks et al., J. Mol. Biol., 222: 581 (1991); Clackson et al.,

30 Nature, 352: (15 August 1991); and Mullinax et al., PNAS (USA), 87: 8095 (Oct. 1990).]

Descriptions of preparing antibodies, which term is herein defined to include biologically active antibody fragments, by recombinant techniques can be found in U.S.

35 Patent No. 4,816,567 (issued March 28, 1989); European Patent Application Publication Number (EP) 338,745 (published Oct. 25, 1989); EP 368,684 (published June 16, 1990); EP 239,400 (published September 30, 1987); WO 90/14424 (published Nov.

29, 1990); WO 90/14430 (published May 16, 1990); Huse et al.,
Science, 246: 1275 (Dec. 8, 1989); Marks et al.,
BioTechnology, 10: 779 (July 1992); La Sastry et al., PNAS
(USA), 86: 5728 (August 1989); Chiang et al., BioTechniques,
5 7(40): 360 (1989); Orlandi et al., PNAS (USA), 86: 3833 (May
1989); Ward et al. Nature, 341: 544 (October 12, 1989);
Marks et al., J. Mol. Biol., 222: 581 (1991); and Hoogenboom
et al., Nucleic Acids Res., 19(15): 4133 (1991).

Representative Mabs

10   Monoclonal antibodies for use in the assays of this
invention may be obtained by methods well known in the art for
example, Galfre and Milstein, "Preparation of Monoclonal
Antibodies: Strategies and Procedures," in Methods in
Enzymology: Immunochemical Techniques, 73: 1-46 [Langone and
15 Vanatis (eds); Academic Press (1981)]; and in the classic
reference, Milstein and Kohler, Nature, 256: 495-497 (1975).]
          Although representative hybridomas of this invention
are formed by the fusion of murine cell lines, human/human
hybridomas [Olsson et al., PNAS (USA), 77: 5429 (1980)] and
20 human/murine hybridomas [Schlom et al., PNAS (USA), 77: 6841
(1980); Shearman et al. J. Immunol., 146: 928-935 (1991); and
Gorman et al., PNAS (USA), 88: 4181-4185 (1991)] can also be
prepared among other possiblities. Such humanized monoclonal
antibodies would be preferred monoclonal antibodies for
25 therapeutic and imaging uses.
          Monoclonal antibodies specific for this invention
can be prepared by immunizing appropriate mammals, preferably
rodents, more preferably rabbits or mice, with an appropriate
immunogen, for example, MaTu-infected HeLa cells, MN fusion
30 proteins, or MN proteins/polypeptides attached to a carrier
protein if necessary. Exemplary methods of producing
antibodies of this invention are described below.
          The monoclonal antibodies useful according to this
invention to identify MN proteins/polypeptides can be labeled
35 in any conventional manner, for example, with enzymes such as
horseradish peroxidase (HRP), fluorescent compounds, or with
radioactive isotopes such as, ^{125}I, among other labels. A

preferred label, according to this invention is ^{125}I, and a
preferred method of labeling the antibodies is by using
chloramine-T [Hunter, W.M., "Radioimmunoassay," In: Handbook
of Experimental Immunology, pp. 14.1-14.40 (D.W. Weir ed.;
5   Blackwell, Oxford/London/Edinburgh/Melbourne; 1978)].

        Representative mabs of this invention include Mabs
M75, MN9, MN12 and MN7 described below. Monoclonal antibodies
of this invention serve to identify MN proteins/polypeptides
in various laboratory diagnostic tests, for example, in tumor
10 cell cultures or in clinical samples.

Mabs Prepared Against HeLa Cells

        MAb M75. Monoclonal antibody M75 (MAb M75) is
produced by mouse lymphocytic hybridoma VU-M75, which was
initially deposited in the Collection of Hybridomas at the
15 Institute of Virology, Slovak Academy of Sciences (Bratislava,
Czechoslovakia) and was deposited under ATCC Designation HB
11128 on September 17, 1992 at the American Type Culture
Collection (ATCC) in Rockville, MD (USA). The production of
hybridoma VU-M75 is described in Zavada et al., WO 93/18152.
20   Mab M75 recognizes both the nonglycosylated GEX-3X-
MN fusion protein and native MN protein as expressed in CGL3
cells equally well. Mab M75 was shown by epitope mapping to
be reactive with the epitope represented by the amino acid
sequence from AA 62 to AA 67 [SEQ. ID. NO.: 10] of the MN
25 protein shown in Figure 1.

Mabs Prepared Against Fusion Protein GEX-3X-MN

        Monoclonal antibodies of this invention were also
prepared against the MN glutathione S-transferase fusion
protein (GEX-3X-MN). BALB/C mice were immunized
30 intraperitoneally according to standard procedures with the
GEX-3X-MN fusion protein in Freund's adjuvant. Spleen cells
of the mice were fused with SP/20 myeloma cells [Milstein and
Kohler, supra].

        Tissue culture media from the hybridomas were
35 screened against CGL3 and CGL1 membrane extracts in an ELISA
employing HRP labelled-rabbit anti-mouse. The membrane

extracts were coated onto microtiter plates. Selected were
antibodies reacted with the CGL3 membrane extract. Selected
hybridomas were cloned twic by limiting dilution.

5 The mabs prepared by the just described method were
characterized by Western blots of the GEX-3X-MN fusion
protein, and with membrane extracts from the CGL1 and CGL3
cells. Representative of the mabs prepared are Mabs MN9, MN12
and MN7.

Mab MN9. Monoclonal antibody MN9 (Mab MN9) reacts
10 to the same epitope as Mab M75, represented by the sequence
from AA 62 to AA 67 [SEQ. ID. NO.: 10] of the Figure 1 MN
protein. As Mab M75, Mab MN9 recognizes both the GEX-3X-MN
fusion protein and native MN protein equally well.

Mabs corresponding to Mab MN9 can be prepared
15 reproducibly by screening a series of mabs prepared against an
MN protein/polypeptide, such as, the GEX-3X-MN fusion protein,
against the peptide representing the epitope for Mabs M75 and
MN9, that is, SEQ. ID. NO.: 10. Alternatively, the Novatope
system [Novagen] or competition with the deposited Mab M75
20 could be used to select mabs comparable to Mabs M75 and MN9.

Mab MN12. Monoclonal antibody MN12 (Mab MN12) is
produced by the mouse lymphocytic hybridoma MN 12.2.2 which
was deposited under ATCC Designation HB 11647 on June 9, 1994
at the American Type Culture Collection (ATCC) at 12301
25 Parklawn Drive, Rockville, MD 20852 (USA). Antibodies
corresponding to Mab MN12 can also be made, analogously to the
method outlined above for Mab MN9, by screening a series of
antibodies prepared against an MN protein/polypeptide, against
the peptide representing the epitope for Mab MN12. That
30 peptide is AA 55 - AA 60 of Figure 1 [SEQ. ID. NO.: 11]. The
Novatope system could also be used to find antibodies specific
for said epitope.

Mab MN7. Monoclonal antibody MN7 (Mab MN7) was
selected from mabs prepared against nonglycosylated GEX-3X-MN
35 as described above. It recognizes the epitope on MN
represented by the amino acid sequence from AA 127 to AA 147
[SEQ. ID. NO.: 12] of the Figure 1 MN protein. Analogously
to methods described above for Mabs MN9 and MN12, mabs

corresponding to Mab MN7 can be prepared by selecting mabs
prepared against an MN protein/polypeptide that are reactive
with the peptide having SEQ. ID. NO.: 12, or by the stated
alternative means.

5 Epitope Mapping

Epitope mapping was performed by the Novatope
system, a kit for which is commercially available from
Novagen, Inc. [See, for analogous example, Li et al., Nature,
363: 85-88 (6 May 1993).] In brief, the MN cDNA was cut into
10 overlapping short fragments of approximately 60 base pairs.
The fragments were expressed in E. coli, and the E. coli
colonies were transferred onto nitrocellulose paper, lysed and
probed with the mab of interest. The MN cDNA of clones
reactive with the mab of interest was sequenced, and the
15 epitopes of the mabs were deduced from the overlapping
polypeptides found to be reactive with each mab.

Therapeutic Use of MN-Specific Antibodies

The MN-specific antibodies of this invention,
monoclonal and/or polyclonal, preferably monoclonal, and as
20 outlined above, may be used therapeutically in the treatment
of neoplastic and/or pre-neoplastic disease, either alone or
in combination with chemotherapeutic drugs or toxic agents,
such as ricin A. Further preferred for therapeutic use would
be biologically active antibody fragments as described herein.
25 Also preferred MN-specific antibodies for such therapeutic
uses would be humanized monoclonal antibodies.

The MN-specific antibodies can be administered in a
therapeutically effective amount, preferably dispersed in a
physiologically acceptable, nontoxic liquid vehicle.

30 Imaging Use of Antibodies

Further, the MN-specific antibodies of this
invention when linked to an imaging agent, such as a
radionuclide, can be used for imaging. Biologically active
antibody fragments or humanized monoclonal antibodies, may be
35 preferred for imaging use.

A patient's neoplastic tissue can be identified as,
for example, sites of transformed stem cells, of tumors and
locations of any metastases. Antibodies, appropriately
labeled or linked to an imaging agent, can be injected in a
5   physiologically acceptable carrier into a patient, and the
binding of the antibodies can be detected by a method
appropriate to the label or imaging agent, for example, by
scintigraphy.

Antisense MN Nucleic Acid Sequences

10   MN genes are herein considered putative oncogenes
and the encoded proteins thereby are considered to be putative
oncoproteins. Antisense nucleic acid sequences substantially
complementary to mRNA transcribed from MN genes, as
represented by the antisense oligodeoxynucleotides ODN1 and
15   ODN2 [SEQ. ID. NOS.: 3 and 4] can be used to reduce or
prevent expression of the MN gene. [Zamecnick, P.C.,
"Introduction: Oligonucleotide Base Hybridization as a
Modulator of Genetic Message Readout," pp. 1-6, Prospects for
Antisense Nucleic Acid Therapy of Cancer and AIDS, (Wiley-
20   Liss, Inc., New York, NY, USA; 1991); Wickstrom, E.,
"Antisense DNA Treatment of HL-60 Promyelocytic Leukemia
Cells: Terminal Differentiation and Dependence on Target
Sequence," pp. 7-24, id.; Leserman et al., "Targeting and
Intracellular Delivery of Antisense Oligonucleotides
25   Interfering with Oncogene Expression," pp. 25-34, id.;
Yokoyama, K., "Transcriptional Regulation of c-myc Proto-
oncogene by Antisense RNA," pp. 35-52, id.; van den Berg et
al., "Antisense fos Oligodeoxyribonucleotides Suppress the
Generation of Chromosomal Aberrations," pp. 63-70, id.;
30   Mercola, D., "Antisense fos and fun RNA," pp. 83-114, id.;
Inouye, Gene, 72: 25-34 (1988); Miller and Ts'o, Ann. Reports
Med. Chem., 23: 295-304 (1988); Stein and Cohen, Cancer Res.,
48: 2659-2668 (1988); Stevenson and Inversen, J. Gen. Virol.,
70: 2673-2682 (1989); Goodchild, "Inhibition of Gene
35   Expression by Oligonucleotides," pp. 53-77,
Oligodeoxynucleotides: Antisense Inhibitors of Gene
Expression (Cohen, J.S., ed; CRC Press, Boca Raton, Florida,

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USA; 1989); Dervan et al., "Oligonucleotide Recognition of
Double-helical DNA by Triple-helix Formation," pp. 197-210,
id.; Neckers, L.M., "Antisense Oligodeoxynucleotides as a Tool
for Studying Cell Regulation: Mechanisms of Uptake and
5   Application to the Study of Oncogene Function," pp. 211-232,
id.; Leitner et al., PNAS (USA), 87: 3430-3434 (1990);
Bevilacqua et al., PNAS (USA), 85: 831-835 (1988); Loke et
al. Curr. Top. Microbiol. Immunol., 141: 282-288 (1988);
Sarin et al., PNAS (USA), 85: 7448-7451 (1988); Agrawal et
10 al., "Antisense Oligonucleotides: A Possible Approach for
Chemotherapy and AIDS," International Union of Biochemistry
Conference on Nucleic Acid Therapeutics (Jan. 13-17, 1991;
Clearwater Beach, Florida, USA); Armstrong, L., Ber. Week, pp.
88-89 (March 5, 1990); and Weintraub et al., Trends, 1: 22-25
15 (1985).] Such antisense nucleic acid sequences, preferably
oligonucleotides, by hybridizing to the MN mRNA, particularly
in the vicinity of the ribosome binding site and translation
initiation point, inhibits translation of the mRNA. Thus, the
use of such antisense nucleic acid sequences may be considered
20 to be a form of cancer therapy.

       Preferred antisense oligonucleotides according to
this invention are gene-specific ODNs or oligonucleotides
complementary to the 5' end of MN mRNA. Particularly
preferred are the 29-mer ODN1 and 19-mer ODN2 [SEQ. ID. NOS.:
25 3 and 4]. Those antisense ODNs are representative of the many
antisense nucleic acid sequences that can function to inhibit
MN gene expression. Ones of ordinary skill in the art could
determine appropriate antisense nucleic acid sequences,
preferably antisense oligonucleotides, from the nucleic acid
30 sequences of Figures 1 and 3a-d.

       Also, as described above, CGL3 cells transfected
with an "antisense" MN cDNA/promoter construct formed colonies
much smaller than control CGL3 cells.

Vaccines

35   It will be readily appreciated that MN proteins and
polypeptides of this invention can be incorporated into
vaccines capable of inducing protective immunity against

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neoplastic disease and a dampening effect upon tumorigenic activity. Efficacy of a representative MN fusion protein GEX-3X-MN as a vaccine in a rat model is shown in Example 2.

5   MN proteins and/or polypeptides may be synthesized or prepared recombinantly or otherwise biologically, to ne comprise one or more amino acid sequences corresponding to or more epitopes of the MN proteins either in monomeric or multimeric form. Those proteins and/or polypeptides may then

10   be incorporated into vaccines capable of inducing protective immunity. Techniques for enhancing the antigenicity of such polypeptides include incorporation into a multimeric structure, binding to a highly immunogenic protein carrier, for example, keyhole limpet hemocyanin (KLH), or diptheria

15   toxoid, and administration in combination with adjuvants or any other enhancers of immune response.

       Preferred MN proteins/polypeptides to be used in a vaccine according to this invention would be genetically engineered MN proteins. Preferred recombinant MN protein are

20   the GEX-3X-MN, MN 20-19, MN-Fc and MN-PA proteins. Other exemplary vaccines include vaccinia-MN (live vaccinia virus with full-length MN cDNA), and baculovirus-MN (full length MN cDNA inserted into baculovirus vector, e.g. in suspension of infected insect cells). Different vaccines may be combined and vaccination periods can be prolonged.

25   A preferred exemplary use of such a vaccine of this invention would be its administration to patients whose MN-carrying primary cancer had been surgically removed. The vaccine may induce active immunity in the patients and prevent recidivism or metastasis.

30   It will further be appreciated that anti-idiotype antibodies to antibodies to MN proteins/polypeptides are also useful as vaccines and can be similarly formulated.

        An amino acid sequence corresponding to an epitope of an MN protein/polypeptide either in monomeric or multimeric form may also be obtained by chemical synthetic means or by

35   purification from biological sources including genetically modified microorganisms or their culture media. [See Lerner, "Synthetic Vaccines", Sci. Am. 248(2): 66-74 (1983).] The

protein/polypeptide may be combined in an amino acid sequence
with other proteins/polypeptides including fragments of other
proteins, as for example, when synthesized as a fusion
protein, or linked to other antigenic or non-antigenic
5   polypeptides of synthetic or biological origin. In some
instances, it may be desirable to fuse a MN protein or
polypeptide to an immunogenic and/or antigenic protein or
polypeptide, for example, to stimulate efficacy of a MN-based
vaccine.

10   The term "corresponding to an epitope of an MN
protein/polypeptide" will be understood to include the
practical possibility that, in some instances, amino acid
sequence variations of a naturally occurring protein or
polypeptide may be antigenic and confer protective immunity
15   against neoplastic disease and/or anti-tumorigenic effects.
Possible sequence variations include, without limitation,
amino acid substitutions, extensions, deletions, truncations,
interpolations and combinations thereof. Such variations fall
within the contemplated scope of the invention provided the
20   protein or polypeptide containing them is immunogenic and
antibodies elicited by such a polypeptide or protein cross-
react with naturally occurring MN proteins and polypeptides to
a sufficient extent to provide protective immunity and/or
anti-tumorigenic activity when administered as a vaccine.

25   Such vaccine compositions will be combined with a
physiologically acceptable medium, including immunologically
acceptable diluents and carriers as well as commonly employed
adjuvants such as Freund's Complete Adjuvant, saponin, alum,
and the like. Administration would be in immunologically
30   effective amounts of the MN proteins or polypeptides,
preferably in quantities providing unit doses of from 0.01 to
10.0 micrograms of immunologically active MN protein and/or
polypeptide per kilogram of the recipient's body weight.
Total protective doses may range from 0.1 to about 100
35   micrograms of antigen. Routes of administration, antigen
dose, number and frequency of injections are all matters of
optimization within the scope of the ordinary skill in the
art.

The following examples are for purposes of illustration only and not meant to limit the invention in any way.

Example 1
Immunohistochemical Staining of Tissue Specimens

To study and evaluate the tissue distribution range and expression of MN proteins, the monoclonal antibody M75 was used to stain immunohistochemically a variety of human tissue specimens. The primary antibody used in these immunohistochemical staining experiments was the M75 monoclonal antibody. A biotinylated second antibody and streptavidin-peroxidase were used to detect the M75 reactivity in sections of formalin-fixed, paraffin-embedded tissue samples. A commercially available amplification kit, specifically the DAKO LSAB™ kit [DAKO Corp., Carpinteria, CA (USA)] which provides matched, ready made blocking reagent, secondary antibody and steptavidin-horseradish peroxidase was used in these experiments.

M75 immunoreactivity was tested according to the methods of this invention in multiple-tissue sections of breast, colon, cervical, lung and normal tissues. Such multiple-tissue sections were cut from paraffin blocks of tissues called "sausages" that were purchased from the City of Hope [Duarte, CA (USA)]. Combined in such a multiple-tissue section were normal, benign and malignant specimens of a given tissue; for example, about a score of tissue samples of breast cancers from different patients, a similar number of benign breast tissue samples, and normal breast tissue samples would be combined in one such multiple-breast-tissue section. The normal multiple-tissue sections contained only normal tissues from various organs, for example, liver, spleen, lung, kidney, adrenal gland, brain, prostate, pancreas, thyroid, ovary, and testis.

Also screened for MN gene expression were multiple individual specimens from cervical cancers, bladder cancers, renal cell cancers, and head and neck cancers. Such specimens were obtained from U.C. Davis Medical Center in Sacramento, CA

and from Dr. Shu Y. Liao [Department of Pathology; St. Joseph Hospital; Orange, CA (USA)].

Controls used in these experiments were the cell lines CGL3 (H/F-T hybrid cells) and CGL1 (H/F-N hybrid cells)
5 which are known to stain respectively, positively and negatively with the M75 monoclonal antibody. The M75 monoclonal antibody was diluted to a 1:5000 dilution wherein the diluent was either PBS [0.05 M phosphate buffered saline (0.15 M NaCl), pH 7.2-7.4] or PBS containing 1% protease-free
10 BSA as a protein stabilizer.

Immunohistochemical Staining Protocol

The immunohistochemical staining protocol was followed according to the manufacturer's instructions for the DAKO LSAB™ kit. In brief, the sections were dewaxed,
15 rehydrated and blocked to remove non-specific reactivity as well as endogenous peroxidase activity. Each section was then incubated with dilutions of the M75 monoclonal antibody. After the unbound M75 was removed by rinsing the section, the section was sequentially reacted with a biotinylated antimouse
20 IgG antibody and streptavidin conjugated to horseradish peroxidase; a rinsing step was included between those two reactions and after the second reaction. Following the last rinse, the antibody-enzyme complexes were detected by reaction with an insoluble chromogen (diaminobenzidine) and hydrogen
25 peroxide. A positive result was indicated by the formation of an insoluble reddish-brown precipitate at the site of the primary antibody reaction. The sections were then rinsed, counterstained with hematoxylin, dehydrated and cover slipped. Then the sections were examined using standard light
30 microscopy.

Interpretation. A deposit of a reddish brown precipitate over the plasma membrane was taken as evidence that the M75 antibody had bound to a MN antigen in the tissue. The known positive control (CGL3) had to be stained to
35 validate the assay. Section thickness was taken into consideration to compare staining intensities, as thicker

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sections produce greater staining intensity independently of other assay parameters.

Results

Preliminary examination of cervical specimens showed that 62 of 68 squamous cell carcinoma specimens (91.2%) stained positively with M75. Additionally, 2 of 6 adenocarcinomas and 2 of 2 adenosquamous cancers of the cervix also stained positively. In early studies, 55.6% (10 of 18) of cervical dysplasias stained positively. A total of 9 specimens including both cervical dysplasias and tumors, exhibited some MN expression in normal appearing areas of the endocervical glandular epithelium, usually at the basal layer. In some specimens, whereas morphologically normal-looking areas showed expression of MN antigen, areas exhibiting dysplasia and/or malignancy did not show MN expression.

M75 positive immunoreactivity was most often localized to the plasma membrane of cells, with the most apparent stain being present at the junctions between adjacent cells. Cytoplasmic staining was most often used as the however, plasma membrane staining was also evident in some main criterion of positivity.

M75 positive cells tended to be near areas showing keratin differentiation in cervical specimens. In some specimens, positive staining cells were located in the center of nests of non-staining cells. Often, there was very little, if any, obvious morphological difference between staining cells and non-staining cells. In some specimens, the positive staining cells were associated with adjacent areas of necrosis.

In most of the squamous cell carcinomas of the cervix, the M75 immunoreactivity was focal in distribution, i.e., only certain areas of the specimen stained. Although the distribution of positive reactivity within a given specimen was rather sporadic, the intensity of the reactivity was usually very strong. In most of the adenocarcinomas of the cervix, the staining pattern was more homogeneous, with the majority of the specimen staining positively.

Among the normal tissue samples, intense, positive
and specific M75 immunoreactivity was observed only in normal
stomach tissues, with diminishing reactivity in the small
intestine, appendix and colon. No other normal tissue stained
5   extensively positively for M75. Occasionally, however, foci
of intensely staining cells were observed in normal intestine
samples (usually at the base of the crypts) or were sometimes
seen in morphologically normal appearing areas of the
epithelium of cervical specimens exhibiting dysplasia and/or
10 malignancy. In such, normal appearing areas of cervical
specimens, positive staining was seen in focal areas of the
basal layer of the ectocervical epithelium or in the basal
layer of endocervical glandular epithelium. In one normal
specimen of human skin, cytoplasmic MN staining was observed
15 in the basal layer. The basal layers of these epithelia are
usually areas of proliferation, suggesting the MN expression
may be involved in cellular growth. In a few cervical
biopsied specimens, MN positivity was observed in the
morphologically normal appearing stratified squamous
20 epithelium, sometimes associated with cells undergoing
koilocytic changes.

Some colon adenomas (4 of 11) and adenocarcinomas (9
of 15) were positively stained. One normal colon specimen was
positive at the base of the crypts. Of 15 colon cancer
25 specimens, 4 adenocarcinomas and 5 metastatic lesions were MN
positive. Fewer malignant breast cancers (3 of 25) and
ovarian cancer specimens (3 of 15) were positively stained.
Of 4 head and neck cancers, 3 stained very intensely with M75.

Although normal stomach tissue was routinely
30 positive, 4 adenocarcinomas of the stomach were MN negative.
Of 3 bladder cancer specimens (1 adenocarcinoma, 1 non-
papillary transitional cell carcinoma, and 1 squamous cell
carcinoma), only the squamous cell carcinoma was MN positive.
Approximately 40% (12 of 30) of lung cancer specimens were
35 positive; 2 of 4 undifferentiated carcinomas; 3 of 8
adenocarcinomas; 2 of 8 oat cell carcinomas; and, 5 of 10
squamous cell carcinomas. One hundred percent (4 of 4) of the
renal cell carcinomas were MN positive.

In summary, MN antigen, as detected by M75 and
immunohistochemistry in the experiments described above, was
shown to be prevalent in tumor cells, most notably in tissues
of cervical cancers. MN antigen was also found in some cells
5   of normal tissues, and sometimes in morphologically normal
appearing areas of specimens exhibiting dysplasia and/or
malignancy. However, MN is not usually extensively expressed
in most normal tissues, except for stomach tissues where it is
extensively expressed and in the tissues of the lower
10   gastrointestinal tract where it is less extensively expressed.
MN expression is most often localized to the cellular plasma
membrane of tumor cells and may play a role in intercellular
communication or cell adhesion. Representative results of
experiments performed as described above are tabulated in
15   Table 2.

TABLE 2
Immunoreactivity of M75 in Various Tissues

	TISSUE	TYPE	POS/NEG (#pos/#tested)
5	liver, spleen, lung, kidney, adrenal gland, brain, prostate, pancreas, thyroid, ovary, testis	normal	NEG (all)
	skin	normal	POS (in basal
10			layer) (1/1)
	stomach	normal	POS
	small intestine	normal	POS
	colon	normal	POS
	breast	normal	NEG (0/10)
15	cervix	normal	NEG (0/2)
	breast	benign	NEG (0/17)
	colon	benign	POS (4/11)
	cervix	benign	POS (10/18)
	breast	malignant	POS (3/25)
20	colon	malignant	POS (9/15)
	ovarian	malignant	POS (3/15)
	lung	malignant	POS (12/30)
	bladder	malignant	POS (1/3)
	head & neck	malignant	POS (3/4)
25	kidney	malignant	POS (4/4)
	stomach	malignant	NEG (0/4)
	cervix	malignant	POS (62/68)

      The results recorded in this example indicate that
the presence of MN proteins in a tissue sample from a patient
30 may, in general, depending upon the tissue involved, be a
marker signaling that a pre-neoplastic or neoplastic process
is occurring. Thus, one may conclude from these results that
diagnostic/prognostic methods that detect MN antigen may be
particularly useful for screening patient samples for a number
35 of cancers which can thereby be detected at a pre-neoplastic
stage or at an early stage prior to obvious morphologic

changes associated with dysplasia and/or malignancy being evident or being evident on a widespread basis.

Example 2

Vaccine -- Rat Model

As shown above in Example 7 of WO 93/18152 (International Publication Date: 16 September 1993), in some rat tumors, for example, the XC tumor cell line (cells from a rat rhabdomyosarcoma), a rat MN protein, related to human MN, is expressed. Thus a model was afforded to study antitumor immunity induced by experimental MN-based vaccines. The following representative experiments were performed.

Nine- to eleven-day-old Wistar rats from several families were randomized, injected intraperitoneally with 0.1 ml of either control rat sera (the C group) or with rat serum against the MN fusion protein GEX-3X-MN (the IM group). Simultaneously both groups were injected subcutaneously with 10^6 XC tumor cells.

Four weeks later, the rats were sacrificed, and their tumors weighed. The results are shown in Figure 2. Each point on the graph represents a tumor from one rat. The difference between the two groups -- C and IM -- was significant by Mann-Whitney rank test (U = 84, α < 0.025). The results indicate that the IM group of baby rats developed tumors about one-half the size of the controls, and 5 of the 18 passively immunized rats developed no tumor at all, compared to 1 of 18 controls.

Example 3

Expression of Full-Length MN cDNA in NIH 3T3 Cells

The role of MN in the regulation of cell proliferation was studied by expressing the full-length cDNA in NIH 3T3 cells. That cell line was chosen since it had been used successfully to demonstrate the phenotypic effect of a number of proto-oncogenes [Weinberg, R.A., Cancer Res., 49: 3713 (1989); Hunter, T., Cell, 64: 249 (1991)]. Also, NIH 3T3 cells express no endogenous MN-related protein that is detectable by Mab M75.

The full length MN cDNA was obtained by ligation of the two cDNA clones using the unique BamHI site and subcloned from pBluescript into KpnI-SacI sites of the expression vector pSG5C. pSG5C was kindly provided by Dr. Richard Kettman

5 [Department of Molecular Biology, Faculty of Agricultural Sciences, B-5030 Gembloux, Belgium]. pSG5C was derived from pSG5 [Stratagene] by inserting a polylinker consisting of a sequence having several neighboring sites for the following restriction enzymes: EcoRI, XhoI, KpnI, BamHI, SacI, 3 times

10 TAG stop codon and BglII.

The recombinant pSG5C-MN plasmid was co-transfected in a 10:1 ratio (10 μg : 1 μg) with the pSV2neo plasmid [Southern and Berg, J. Mol. Appl. Genet., 1: 327 (1982)] which contains the neo gene as a selection marker. The co-

15 transfection was carried out by calcium phosphate precipitation method [Mammalian Transfection Kit; Stratagene] into NIH 3T3 cells plated a day before at a density of 1×10^5 per 60 mm dish. As a control, pSV2neo was co-transfected with empty pSG5C.

20 Transfected cells were cultured in DMEM medium supplemented with 10% FCS and 600 μg ml^{-1} of G418 [Gibco BRL] for 14 days. The G418-resistant cells were clonally selected, expanded and analysed for expression of the transfected cDNA by Western blotting using iodinated Mab M75.

25 For an estimation of cell proliferation, the clonal cell lines were plated in triplicates (2×10^4 cells/well) in 24-well plates and cultivated in DMEM with 10% FCS and 1% FCS, respectively. The medium was changed each day, and the cell number was counted using a hemacytometer.

30 To determine the DNA synthesis, the cells were plated in triplicate in 96-well plate at a density of 10^4/well in DMEM with 10% FCS and allowed to attach overnight. Then the cells were labeled with ^{3}H-thymidine for 24 hours, and the incorporated radioactivity was counted.

35 For the anchorage-independent growth assay, cells (2×10^4) were suspended in a 0.3% agar in DMEM containing 10% FCS and overlaid onto 0.5% agar medium in 60 mm dish. Colonies grown in soft agar were counted two weeks after plating.

Several clonal cell lines constitutively expressing both 54 and 58 kd forms of MN protein in levels comparable to those found in LCMV-infected HeLa cells were obtained. Selected MN-positive clones and negative control cells (mock-transfected with an empty pSG5C plasmid) were subjected to further analyses directed to the characterization of their phenotype and growth behavior.

The MN-expressing NIH 3T3 cells displayed spindle-shaped morphology, and increased refractility; they were less adherent to the solid support and smaller in size. The control (mock transfected cells) had a flat morphology, similar to parental NIH 3T3 cells. In contrast to the control cells that were aligned and formed a monolayer with an ordered pattern, the cells expressing MN lost the capacity for growth arrest and grew chaotically on top of one another. Correspondingly, the MN-expressing cells were able to reach significantly higher (more than 2x) saturation densities (Table 3) and were less dependent on growth factors than the control cells.

MN transfectants also showed faster doubling times (by 15%) and enhanced DNA synthesis (by 10%), as determined by the amount of [^{3}H]-thymidine incorporated in comparison to control cells. Finally, NIH 3T3 cells expressing MN protein grew in soft agar. The diameter of colonies grown for 14 days ranged from 0.1 to 0.5 mm; however, the cloning efficiency of MN transfectants was rather low (2.4%). Although that parameter of NIH 3T3 cells seems to be less affected by MN than by conventional oncogenes, all other data are consistent with the idea that MN plays a role in cell growth control.

Table 3

Growth Properties of NIH 3T3 Cells Expressing MN Protein

Transfected DNA	pSG5C/ pSV2neo	pSG5C-MN/ pSV2neo
Doubling time[a] (hours)	27.9 ± 0.5	24.1 ± 1.3

Saturation density[b] (cells x $10^4/cm^2$)	4.9 ± 0.2	11.4 ± 0.4
Cloning efficiency (%)[c]	< 0.01	2.4 ± 0.2

_____

[a]For calculation of the doubling time, the proliferation rate
of exponentially growing cells was used. [b]The saturation cell
density was derived from the cell number 4 days after reaching
confluency. [c]Colonies greater than 0.1 mm in diameter were
scored at day 14. Cloning efficiency was estimated as a
percentage of colonies per number of cells plated, with
correction for cell viability.

Example 4
Acceleration of G1 Transit and Decrease in Mitomycin C
Sensitivity Caused by MN Protein

     For the experiments described in this example, the
stable MN transfectants of NIH 3T3 cells generated as
described in Example 3 were used. Four selected MN-positive
clones and four control mock-transfected clones were either
used individually or in pools.
     Flow cytometric analyses of asynchronous cell
populations. Cells that had been grown in dense culture were
plated at 1 x 10^6 cells per 60 mm dish. Four days later, the
cells were collected by trypsinization, washed, resuspended in
PBS, fixed by dropwise addition of 70% ethanol and stained by
propidium iodine solution containing RNase. Analysis was
performed by FACStar using DNA cell cycle analysis software
[Becton Dickinson; Franklin Lakes, NJ (USA)].
     Exponentially growing cells were plated at 5 x 10^5
cells per 60 mm dish and analysed as above 2 days later.
Forward light scatter was used for the analysis of relative
cell sizes. The data were evaluated using Kolmogorov-Smirnov
test [Young, _J. Histochem. Cytochem., 25_: 935 (1977)].
     The flow cytometric analyses revealed that clonal
populations constitutively expressing MN protein showed a

decreased percentage of cells in G1 phase and an increased
percentage of cells in G2-M phases. Those differences were
more striking in cell populations grown throughout three
passages in high density cultures than in exponentially

5 growing subconfluent cells. That observation supports the
idea that MN protein has the capacity to perturb contact
inhibition.

        Also observed was a decrease in the size of MN
expressing cells seen in both exponentially proliferating and

10 high density cultures. It is possible that the MN-mediated
acceleration of G1 transit is related to the above-noted
shorter doubling time (by about 15%) of exponentially
proliferating MN-expressing NIH 3T3 cells. Also, MN
expressing cells displayed substantially higher saturation

15 density and lower serum requirements than the control cells.
Those facts suggest that MN-transfected cells had the capacity
to continue to proliferate despite space limitations and
diminished levels of serum growth factors, whereas the control
cells were arrested in G1 phase.

20   Limiting conditions. The proliferation of MN-
expressing and control cells was studied both in optimal and
limiting conditions. Cells were plated at 2×10^4 per well of
24-well plate in DMEM with 10% FCS. The medium was changed at
daily intervals until day 4 when confluency was reached, and

25 the medium was no longer renewed. Viable cells were counted
in a hemacytometer at appropriate times using trypan blue dye
exclusion. The numbers of cells were plotted versus time
wherein each plot point represents a mean value of triplicate
determination.

30   The results showed that the proliferation of MN
expressing and control cells was similar during the first
phase when the medium was renewed daily, but that a big
difference in the number of viable cells occurred after the
medium was not renewed. More than half of the control cells

35 were not able to withstand the unfavorable growth conditions.
In contrast, the MN-expressing cells continued to proliferate
even when exposed to increasing competition for nutrients and
serum growth factors.

Those results were supported also by flow cytometric
analysis of serum starved cells grown for two days in medium
containing 1% FCS. While 83% of control cells accumulated in
G0-G1 phase (S = 5%, G2-M = 12%), expression of MN protein
5   partially reversed the delay in G1 as indicated by cell cycle
distribution of MN tranfectants (G0-G1 = 65%, S = 10%, G2-M =
26%). The results of the above-described experiments suggest
that MN protein might function to release the G1/S checkpoint
and allow cells to proliferate under unfavorable conditions.

10   MMC. To test that assumption, unfavorable
conditions were simulated by treating cells with the DNA
damaging drug mitomycin C (MMC) and then following their
proliferation and viability. The mechanism of action of MMC
is thought to result from its intracellular activation and
15 subsequent DNA alkylation and crosslinking [Yier and
Szybalski, Science, 145: 55 (1964)]. Normally, cells respond
to DNA damage by arrest of their cell cycle progression to
repair defects and prevent acquisition of genomic instability.
Large damage is accompanied by marked cytotoxicity. However,
20 many studies [for example, Peters et al., Int. J. Cancer, 54:
450 (1993)] concern the emergence of drug resistant cells both
in tumor cell populations and after the introduction of
oncogenes into nontransformed cell lines.

The response of MN-transfected NIH 3T3 cells to
25 increasing concentrations of MMC was determined by continuous
[^{3}H]-thymidine labeling. Cells were plated in 96-well
microtiter plate concentration of 10^4 per well and incubated
overnight in DMEM with 10% FCS to attach. Then the growth
medium was replaced with 100 μl of medium containing
30 increasing concentrations of MMC from 1 μl/ml to 32 μg/ml.
All the drug concentrations were tested in three replicate
wells. After 5 hours of treatment, the MMC was removed, cells
were washed with PBS and fresh growth medium without the drug
was added. After overnight recovery, the fractions of cells
35 that were actively participating in proliferation was
determined by continuous 24-hr labeling with [^{3}H]-thymidine.
The incorporation by the treated cells was compared to that of
the control, untreated cells, and the proliferating fractions

were considered as a percentage of the control's incorporation.

The viability of the treated cells was estimated three days later by a CellTiter 96 AQ Non-Radioactive Cell
5 Proliferation Assay [Promega] which is based on the bioreduction of methotrexate (MTX) into a water soluble formazan that absorbs light at 490 nm. The percentage of . surviving cells was derived from the values of absorbance obtained after substraction of background.
10 The control and MN-expressing NIH 3T3 cells showed remarkable differences in their responses to MMC. The sensitivity of the MN-transfected cells appeared considerably lower than the control's in both sections of the above-described experiments. The results suggested that the MN-
15 transfected cells were able to override the negative growth signal mediated by MMC.

ATCC Deposits. The material listed below was deposited with the American Type Culture Collection (ATCC) at 12301 Parklawn Drive, Rockville, MD 20852 (USA). The
20 deposits were made under the provisions of the Budapest Treaty on the International Recognition of Deposited Microorganisms for the Purposes of Patent Procedure and Regulations thereunder (Budapest Treaty). Maintenance of a viable culture is assured for thirty years from the date of deposit. The
25 hybridomas and plasmids will be made available by the ATCC under the terms of the Budapest Treaty, and subject to an agreement between the Applicants and the ATCC which assures unrestricted availability of the deposited hybridomas and plasmids to the public upon the granting of patent from the
30 instant application. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any Government in accordance with its patent laws.

	Hybridoma	Deposit Date	ATCC #
35	VU-M75	September 17, 1992	HB 11128
	MN 12.2.2	June 9, 1994	HB 11647

Plasmid	Deposit Date	ATCC #
A4a	June 6, 1995	97199
XE1	June 6, 1995	97200
XE3	June 6, 1995	97198

5   The description of the foregoing embodiments of the
invention have been presented for purposes of illustration and
description. They are not intended to be exhaustive or to
limit the invention to the precise form disclosed, and
obviously many modifications and variations are possible in
10   light of the above teachings. The embodiments were chosen and
described in order to explain the principles of the invention
and its practical application to enable thereby others skilled
in the art to utilize the invention in various embodiments and
with various modifications as are suited to the particular use
15   contemplated.
All references cited herein are hereby incorporated
by reference.

CLAIMS

1. An isolated nucleic acid containing at least twenty-seven nucleotides wherein the nucleotide sequence for said nucleic acid is selected from the group consisting of:

     (a) SEQ. ID. NOS.: 1, 5, and 27-49 and nucleotide sequences complementary to SEQ. ID. NOS.: 1, 5 and 27-49;

     (b) nucleotide sequences that hybridize under stringent hybridization conditions to one or more of the following nucleotide sequences: SEQ. ID. NOS.: 1, 5, and 27-49 and the respective complements of SEQ. ID. NOS.: 1, 5 and 27-49; and

     (c) nucleotide sequences that differ from the nucleotide sequences of (a) and (b) in codon sequence due to the degeneracy of the genetic code.

2. An isolated nucleic acid according to Claim 1 wherein said nucleotide sequence is selected from the group consisting of:

     (a) SEQ. ID. NO.: 5 and its complement;

     (b) nucleotide sequences that hybridize under stringent conditions to SEQ. ID. NO.: 5 or to its complement;

     (c) nucleotide sequences that differ from the nucleotide sequences of (a) or (b) in codon sequence due to the degeneracy of the genetic code.

3. An isolated nucleic acid containing at least sixteen nucleotides wherein the nucleotide sequence therefor is selected from the group consisting of:

     (a) the MN nucleotide sequences contained in plasmids A4a, XE1 and XE3 which were deposited at the American Type Culture Collection (ATCC) in Rockville, Maryland in the United State of America under the respective ATCC Nos. 97199, 97200, and 97198;

     (b) nucleotide sequences that hybridize under stringent conditions to the MN nucleotide sequences of (a); and

        (c) nucleotide sequences that differ from the
nucleotide sequences of (a) or (b) in codon sequence due to
the degeneracy of the genetic code.

        4.   An isolated nucleic acid according to Claim 3
5   which functions as a polymerase chain reaction primer for MN
nucleic acid sequences, and which is from 16 to about 50
nucleotides in length.

        5.   An isolated nucleic acid, containing at least
fifty nucleotides, encoding an MN protein or polypeptide that
10 is specifically bound either by monoclonal antibodies
designated M75 secreted by the hybridoma VU-M75 deposited at
the American Type Culture Collection (ATCC) in Rockville,
Maryland in the United States of America under ATCC No. HB
11128, or by monoclonal antibodies designated MN12 secreted by
15 the hybridoma MN 12.2.2 deposited at the ATCC under ATCC No.
11647, or by both of said monoclonal antibodies.

        6.   An isolated nucleic acid which is operatively
linked to an expression control sequence within a vector
wherein said nucleic acid is selected from the group
20 consisting of:
        (a) SEQ. ID. NO.: 1 and its complement;
        (b) nucleic acids that hybridize under stringent
hybridization conditions to SEQ. ID. NO.: 1 or to its
complement; and
25   (c) nucleic acids that differ from the nucleic acid
sequences of (a) and (b) due to the degeneracy of the genetic
code.

        7.   A unicellular host, which is either prokaryotic
or eukaryotic, that is transformed or transfected with the
30 isolated nucleic acid operatively linked to an expression
control sequence in a vector according to Claim 6.

8. A method of recombinantly producing an MN protein, MN fusion protein or MN polypeptide comprising the steps of:

(a) transforming a unicellular host with the
5 isolated nucleic acid operatively linked to an expression control sequence in a vector according to Claim 6;

(b) culturing said unicellular host so that said MN protein or polypeptide is expressed; and

(c) extracting and isolating said MN protein or
10 polypeptide.

9. A recombinant nucleic acid encoding a fusion protein, that consists essentially of an MN protein or polypeptide and a non-MN protein or polypeptide, wherein the nucleotide sequence for the portion of the nucleic acid encoding the MN protein or polypeptide is selected from the
15 group consisting of:

(a) SEQ. ID. NO.: 1;

(b) nucleotide sequences that hybridize under stringent conditions to SEQ. ID. NO.: 1 or to its complement;
20 and

(c) degenerate variants of SEQ. ID. NO.: 1 and of the nucleotide sequences of (b);

wherein the nucleic acid encoding said MN protein or polypeptide contains at least twenty-nine nucleotides.

25 10. A method of detecting mutations in an isolated MN gene and/or fragment(s) thereof comprising the steps of:

amplifying one or more fragment(s) of said gene by the polymerase chain reaction (PCR); and

determining whether said one or more fragments
30 contain any mutations.

11. An MN protein, MN fusion protein or MN polypeptide, wherein said MN protein, MN polypeptide or the MN protein portion of said MN fusion protein is encoded by a nucleic acid of at least twenty-nine nucleotides which is
35 selected from the group consisting of:

      (a) SEQ. ID. NO. NO.: 1;

      (b) nucleotide sequences that hybridize under stringent hybridization conditions to SEQ. ID. NO.: 1 or to its complement; and

      (c) nucleotide sequences that differ from SEQ. ID. NO.: 1 or from the sequences of (b) in codon sequence due to the degeneracy of the genetic code.

      12. An MN protein, MN fusion protein, or MN polypeptide wherein said MN protein or polypeptide has, and wherein said MN fusion protein contains, an amino acid sequence selected from the group consisting of SEQ. ID. NOS.: 2, 6, 10-16, and 50-53.

      13. A vaccine comprising an immunogenic amount of one or more MN proteins, MN fusion proteins, and/or MN polypeptides according to Claims 11 or 12 dispersed in a physiologically acceptable, nontoxic vehicle, which amount is effective to immunize a vertebrate against a neoplastic disease associated with expression of MN antigen.

      14. An antibody which specifically binds to an MN protein, an MN fusion protein and/or an MN polypeptide according to Claims 11 or 12.

      15. A monoclonal antibody according to Claim 14 which is designated MN12 and is secreted by the hybridoma MN 12.2.2 which was deposited at the American Type Culture Collection (ATCC) in Rockville, Maryland in the United States of America under ATCC No. HB 11647.

      16. An antibody according to Claim 14 which specifically binds to an MN antigen epitope selected from the group of epitopes represented by the following amino acid sequences: SEQ. ID. NOS. 10-16.

17. An antibody according to Claim 14 which is linked to an imaging agent, to a chemotherapeutic agent or to a toxic agent.

18. A method of imaging pre-neoplastic or neoplastic disease in a patient comprising:
    (a) injecting said patient with antibody linked to an imaging agent according to Claim 17; and
    (b) detecting the binding of said antibody.

19. A hybridoma designated MN 12.2.2 which produces the monoclonal antibody MN12, and which was deposited at the American Type Culture Collection (ATCC) in Rockville Maryland in the United States of America under ATCC Accession No. HB 11647.

20. A method of delivering a chemotherapeutic agent or toxic agent to a cancer cell which comprises contacting said cell with an antibody linked to a chemotherapeutic agent or to a toxic agent according to Claim 17.

21. A method of treating neoplastic disease in a patient comprising administering to said patient a therapeutically effective amount of a composition comprising antibodies which specifically bind to an MN protein, an MN fusion protein and/or an MN polypeptide according to Claims 11 or 12.

22. A method of detecting and/or quantitating in a vertebrate sample MN antigen comprising the steps of:
    (a) contacting said sample with one or more antibodies according to Claims 14, 15 or 16; and
    (b) detecting and/or quantitating binding of said antibody in said sample.

23. A method according to Claim 22 wherein said vertebrate sample is a human tissue specimen, such as, a cell

smear, a histological section from a biopsied tissue or organ, or an imprint preparation.

24.   A method according to Claim 23 wherein said tissue specimen is ovarian, endometrial, or cervical.

5   25.   A method of detecting and/or quantitating MN-specific antibodies in a vertebrate sample comprising the steps of:
          (a) contacting and incubating the vertebrate sample with an MN protein, an MN fusion protein and/or an MN
10   polypeptide according to Claims 11 or 12; and
          (b) detecting and/or quantitating binding of said MN protein, MN fusion protein and/or MN polypeptide to antibody in said sample.

          26.   A method of treating neoplastic disease and/or
15   pre-neoplastic disease comprising inhibiting the expression of MN genes by administering one or more antisense nucleic acid sequences that hybridize under stringent conditions to mRNA transcribed from MN genes.

          27.   Vectors containing an MN nucleic acid sequence
20   wherein said MN nucleic acid sequence is selected from the group consisting of:
          (a) SEQ. ID. NO.: 5 and its complement;
          (b) nucleic acids that hybridize to SEQ. ID. NO.:   5 or to its complement; and
25   (c) nucleic acids that differ from the nucleic acids of (a) or (b) due to the degeneracy of the genetic code;
          wherein said nucleic acid is at least twenty-nine nucleotides in length.

          28.   Vectors containing an MN nucleic acid sequence
30   according to Claim 27 selected from the group consisting of the plasmids A4a, XE1 and XE3 which are deposited at the American Type Culture Collection (ATCC) in Rockville, Maryland

in the United States of America under the respective ATCC
accession numbers 97199, 97200 and 97198.

```
  1    1                            M   A   P   L   C   P   S   P   W   L   P   L    12
                  ACA GTC AGC CGC ATG GCT CCC CTG TGC CCC AGC CCC TGG CTC CCT CTG    48

 13   49   L   I   P   A   P   A   T   L   G   L   Q   V   L   L   L   S             28
          TTG ATC CCG GCC CCT GCT ACT CTC GGC CTG CAA GTG CTG CTG CTG TCA            96

 29   97   L   L   M   L   P   V   H   P   Q   R   L   P   R   M   M   Q             44
          CTG CTT ATG CTG CCT GTC CAT CCC CAG AGG TTG CCC CGG ATG ATG CAG           144

 45  145   E   D   L   E   S   E   E   D   E   E   E   E   D   P   D   L             60
          GAG GAT CTA GAG TCA GAG GAG GAT GAG GAG GAG GAG GAT CCA GAC CTG           192

 61  193   G   E   D   L   P   S   G   S   G   G   S   E   G   E   E   D             76
          GGC GAG GAT CTG CCC AGT GGA TCT GGA GGC TCT GAA GGG GAG GAG GAT           240

 77  241   P   E   G   E   L   P   G   E   S   P   R   L   P   G   G   E             92
          CCA CCC GGA GAG CTA CCT GGA GAG TCA CCC AGA CTA CCT GGA GGA GAG           288

 93  289   E   D   L   P   E   V   K   P   K   S   E   E   E   E   S   L            108
          GAG GAT CTA CCT GAG GTT AAG CCT AAA TCA GAA GAG GAG GAA TCC CTG           336

109  337   K   L   D   L   P   T   V   E   A   P   G   D   P   D   Q   E            124
          AAG TTA GAG GAT CTA CCT ACT GTT GAG GCT CCT GGA GAT CCT CAA GAA           384

125  385   P   Q   N   N   A   H   R   D   K   E   G   D   D   Q   S   H            140
          CCC CAG AAT AAT GCC CAC AGG GAC AAA GAA GGG GAC GAT CAG AGT CAT           432

141  433   W   R   Y   G   G   D   P   P   P   W   R   V   S   P   A   C            156
          TGG CGC TAT GGA GGC GAC CCG GAC CCC TGG CGG GTG TCC CCA GCC TGC           480

157  461   A   G   R   F   Q   R   R   V   D   I   R   P   Q   L   A   A            172
          GCG GGC CGC TTC CAG CGC CGC GTG GAT ATC CGC CCC CAG CTC GCC GCC           528
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FIG._1A

FIG._1B

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173  F   C   P   A   L   R   P   L   E   L   G   F   Q   L   P                          188
529  TTC TGC CCG GCC CTG CGC CCC CTG GAA CTC GGC TTC CAG CTC CCG                          576

189  P   L   E   P   L   R   L   N   N   G   H   S   V   Q   L                          204
577  CCG CTC GAA CCA CTG CGC CTG AAT AAC GGC CAC AGT GTG CAA CTG                          624

205  T   L   P   G   L   M   A   L   G   P   G   R   R   E   Y                          220
625  ACC CTG CCT GGG CTA ATG GCT CTG GGT CCC GGG CGG CGG GAG TAC                          672

221  R   A   L   Q   L   H   L   H   W   G   A   A   R   P   G                          236
673  CGG GCT CTG CAG CTG CAT CTG CAC TGG GGG GCA GCT CGT CCG GGC                          720

237  S   E   H   T   V   E   G   V   T   A   E   F   P   A   H   V                      252
721  TCG GAG CAC ACT GTG GAA GGC GTG ACT GCC GAG TTC CCT GCC CAC GTG                      768

253  V   H   L   S   T   S   A   F   A   R   V   D   E   A   L   G   R                  268
769  GTT CAC CTC AGC ACC AGC GCC TTT GCC AGA GTT GAC GAG GCC TTG GGG CGC                  816

269  P   G   G   L   A   V   L   A   F   L   E   E   G   E   P   E                      284
817  CCG GGA GGC CTG GCC GTG TTG GCC TTT CTG GAG GAG GGC GAG CCG GAA                      864

285  E   N   S   A   Y   E   Q   L   S   R   L   E   I   A                              300
865  GAA AAC AGT GCC TAT GAG CAG CTG AGT CGC TTG GAA ATC GCT                              912

301  E   E   S   G   E   T   Q   G   V   P   L   D   I   S   A   L                      316
913  GAG GAA TCA GGC GAG ACT CAG GGA GTC CCA CTG GAC ATA TCT GCA CTC                      960

317  L   P   S   D   F   S   R   Y   F   Q   S   G   L   S   T                          332
961  CTG CCC TCT GAC TTC AGC CGC TAC TTC CAA TCT GGG TAT CTG TCT ACT                      1008

333  T   P   P   C   A   Q   G   V   I   W   V   F   N   Q   T                          348
1009 ACA CCG CCC TGT GCC CAG GGT GTC ATC TGG GTG TTT AAC CAG ACA                          1056
```

FIG.–1A
FIG.–1B
FIG.–1C

FIG._1

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349  V   M   L   S   A   K   Q   L   H   T   L   S   D   T   L   W   364
1057 GTG ATG CTG AGT GCT AAG CAG CTC CAC ACC CTC TCT GAC ACC CTG TGG 1104

365  G   P   G   D   S   R   L   Q   L   N   F   R   A   T   Q   P   380
1105 GGA CCT GGT GAC TCT CGG CTA CAG CTG AAC TTC CGA GCG ACG CAG CCT 1152

381  L   N   G   R   V   I   E   S   F   A   P   A   G   V   D   S   396
1153 TTG AAT GGG CGA GTG ATT GAG TCC TTC GCC CCT GCT GGA GTG GAC AGC 1200

397  S   P   R   A   A   E   P   V   Q   L   N   S   C   L   A   A   412
1201 AGT CCT CGG GCT GCT GAG CCA GTC CAG CTG AAT TCC TGC CTG GCT GCT 1248

413  G   D   I   L   A   L   V   F   L   F   G   L   L   A   V   S   428
1249 GGT GAC ATC CTA GCC CTG GTT TTT CTT TTT GGC CTC TTT GCT ACC AGC 1296

429  V   A   F   L   V   Q   M   R   R   Q   H   R   G   R   T   K   444
1297 GTC GCG TTC CTT GTT CAG ATG AGA AGG CAG CAC AGA AGG GGA ACC AAA 1344

445  G   G   V   S   Y   R   P   A   E   V   A   E   T   G   A   *   460
1345 GGG GGT GTG AGC TAC CGC CCA GCA GAG GTA GCC GAG ACT GGA GCC TAG 1392

1393 AGG CTG GAT CTT GGA GAA TGT CCT GTC CTG CTC ATT ATG CCA GGC ATC TGA GGG 1440

1441 GGA GCC GGT AAC TGT CCT GTC CTG CTC ATT ATG CCA CTT CCT TTT AAC 1488

1489 TGC CAA GAA ATT TTT TAA AAT AAA TAT TTA TAA T 1522
```
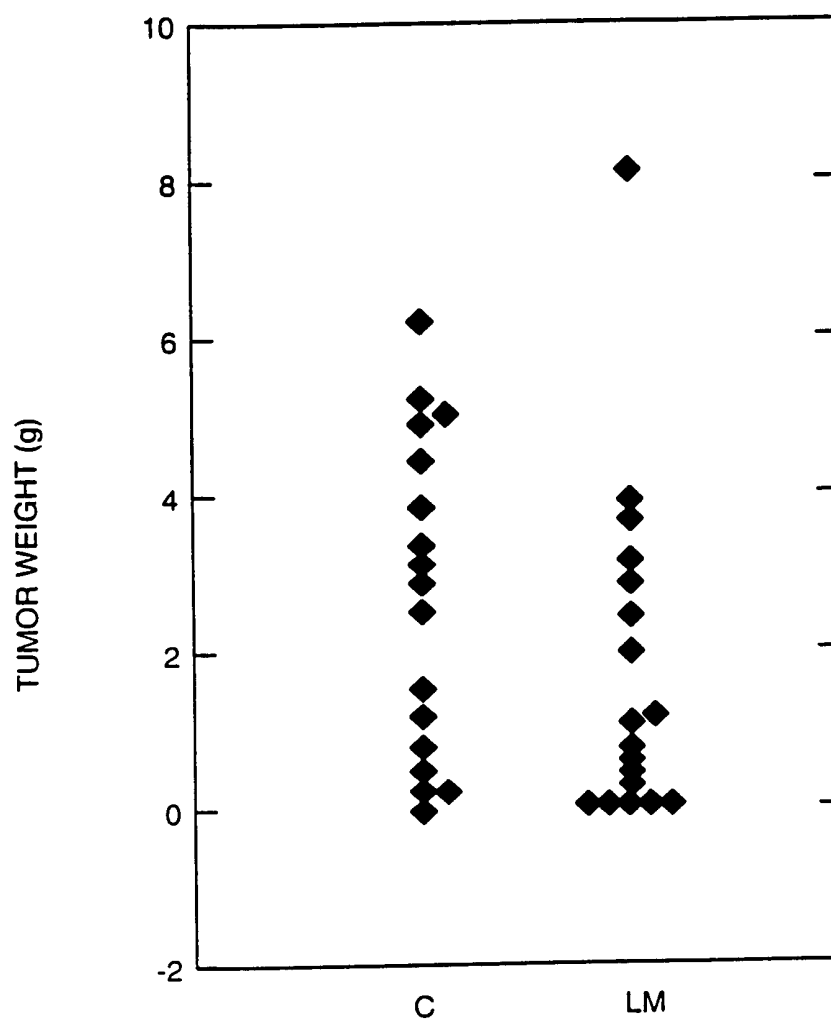
FIG._1C

FIG._2

FIG._3A

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   1 ggatcctgtt gactcgtgac cttacccca acctgtgct ctctgaaaca tgagctgtgt
  61 ccactcaggg ttaaatggat taaggggcggt gcaagatgtg cttgtttaaa cagatgcttg
 121 aaggcagcat gctcgttaag agtcatcacc aatccctaat ctcaagtaat cagggacaca
 181 aacactgcgg aaggccgcag ggtcctctgc ctaggaaaac cagagacctt tgttcacttg
 241 tttatctgac cttcccctcca ctattgtcca tgaccctgcc aaatccccct ctgtgagaaa
 301 cacccaagaa ttatcaataa aaaaataaat ttaaaaaaaa aatacaaaaa aaaaaaaaaa
 361 aaaaaaaaaa gacttacgaa tagttattga taaatgaata gctattggta aagccaagta
 421 aatgatcata ttcaaaacca gacggccatc atcacagctc aagtctacct gatttgatct
 481 cttatcatt gtcattcttt ggattcacta gattagtcat catcctcaaa attctccccc
 541 aagttctaat tacgttccaa acattaggg gttacatgaa gctttgaacct actacccttct
 601 ttgcttttga gccatgagtt gtaggaatga tgagttaca cctacatgc tggggattaa
 661 tttaaactttt acctctaagt cagtgggta gcctttggct tattttgta gctaatttttg
 721 tagttaatgg atgcactgtg aatcttgcta tgatagtttt cctccacact ttgccactag
 781 gggtaggtag gtactcagtt ttcagtaatt gcttacctaa gacccctaagc cctattttctc
 841 ttgtactggc ctttatctgt aatatgggca tatttaatac aatataattt ttggagtttt
 901 tttgttttgtt tgtttgtttg ttttttttgag acggagtctt gcatctgtca tgcccaggct
 961 ggagtagcag tggtgccatc tcggctcact gcaagctcca cctcccgagt tcacgccatt
1021 ttcctgcctc agcctcccga gtagctggga ctacagggcgc ccgccaccat gcccggctaa
1081 tttttgtat tttttggtaga gacgggggtt caccgtgtta gccagaatgg tctcgatctc
1141 ctgacttcgt gatccacccg cctcggcctc ccaaagttct gggattacag gtgtgagcca
1201 ccgcacctgg ccaattttt gagtctttta aagtaaaaat atgtcttgta agctggtaac
1261 tatggtacat ttcctttttat ctgacggtca tataggttct tttgagtttg agctggtttg
1321 gcatgcatat gctactttt gcagtccttt cattacatt tctctcttc attgaagag
1381 catgttatat cttttagctt cacttggctt aaaaggttct ctcattagcc taacacagtg
1441 tcattgttgg taccacttgg atcataagtg gaaaacagt caagaaattg cacagtaata
1501 cttgtttgta agagggatga ttcaggtgaa tctgacacta agaaactccc ctacctgagg
1561 tctgagattc ctctgacatt gctgtatata ggctttcct ttgacagcct gtgactgcgg
1621 actatttttc ttaagcaaga tatgctaaga tttgtgagc ctttttccag agagaggtct
1681 catatctgca tcaagtgaga acatataatg tctgcatgtt tccatatttc aggaatgttt
1741 gcttgtgttt tatgctttta tatagacagg gaaacttgtt cctcagtgac ccaaaagagg
1801 tgggaattgt tattggatat catcattggc ccacgcttc tgaccttgga aacaattaag
1861 ggttcataat ctcaattctg tcagaattgg tacaagaaat agctgctatg ttttttgaca
1921 ttccacttgg taggaaataa gaatgtgaa ctcttcagtt ggtgtgtgtc cct?gtttt
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1981 ttgcaatttc cttcttactg tgttaaaaaa aagtatgatc ttgtctctgag aggtgaggca
2041 ttcttaatca tgatctttaa agatcaataa tataatcctt tcaaggatta tgtctttatt
2101 ataataaaga taatttgtct ttaacagaat caataatata atcccttaaa ggattatatc
2161 tttgctgggc gcagtggctc acacctgtaa tcccagcact ttgggtggcc aaggtggaag
2221 gatcaaattt gcctacttct atattatctt ctaaagcaga attcatctct cttccctcaa
2281 tatgatgata ttgacaggggt ttgccctcac tcactagatt gtgagctcct gctcaggggca
2341 ggtagcgttt tttgtttttg tttttttttt tcttttttga gacaggggtct tgtctgtgtca
2401 cccaggccag agtgcaatgg tacagtctca gctcactgca gcctcaaccg cctcggctca
2461 aaccatcatc ccatttcagc ctcctgagta gctgggacta caggcacatg ccattacacc
2521 tggctaattt ttttgtattt ctagtagaga cagggtttgg ccatgttgcc cgggctggtc
2581 tcgaactcct ggactcaagc aatccaccca cctcagcctc ccaaaatgag ggaccgtgtc
2641 ttattcattt ccatgtccct agtccatagc ccagtgctgg acctatggta gtactaaata
2701 aatatttgtt gaatgcaata gtaaatagca tttcagggag caagaactag attaacaaag
2761 gtgggtaaaag gtttggagaa aaaaataata gtttaatttg gctagagtat gagggagagt
2821 agtaggagac aagatggaaa ggtctcttgg gcaaggtttt gaaggaagtt ggaagtcaga
2881 agtacacaat gtgcatatcg tggcaggcag tggggagcca atgaaggctt ttgagcagga
2941 gagtaatgtg ttgaaaaata aatataggtt aaacctatca gagcccctct gacacataca
3001 cttgctttc attcaagctc aagtttgtct cccacatacc cattacttaa ctcaccctcg
3061 ggctccccta gcagcctgcc ctacctcttt acctgcttcc tggtggagtc aggggatgtat
3121 acatgagctg cttccctct cagccagagg acatgggggg cccagctcc cctgccttc
3181 cccttctgtg cctggagctg ggaagcaggc cagggttagc tgaggctggc tggcaagcag
3241 ctgggtggtg ccaggggagag cctgccatagt gccaggtggt gccttgggtt ccaagctagt
3301 ccatggcccc gataaccttc tgcctgtgca cacacctgcc cctcactcca ccccatcct
3361 agctttggta tgggggagag ggcacagggc cagacaaacc tgtgagactt tggctccatc
3421 tctgcaaaag ggcgctctgt gagtcagcct gctcccctcc agcttgctc ctccccacc
3481 cagctctcgt ttccaatgca cgtacagccc gtacacaccg tgtgctggga caccccACAG
3541 TCAGCCGCAT GGCTCCCCTG TGCCCCAGCC CCTGGCTCCC TCTGTTGATC CCGGCCCCTG
3601 CTCCCAGGCCT CACTGCTGT CTGCTGCTGT CACTGCTGCT TCTGGTGCCT GTCCATCCCC
3661 AGAGGTTGCC CCGGATGCAG GAGGATTCCC CCTTGGGAGG AGGCTCTTCT GGGGAAGATG
3721 ACCCACTGGG CGAGGAGGAT CTGCCCAGTG AAGAGGATTC ACCCAGAGAG GAGGATCCAC
3781 CCGGAGAGGA GGATCTACCT GGAGGAGGAG ATCTACCTGG AGAGGAGGAT CTACCTGAAG
3841 TTAAGCCTAA ATCAGAAGAA GAGGGCTCCC TGAAGTTAGA GGATCTACCT ACTGTTGAGG
3901 CTCCTGGAGA TCCTCAAGAA CCCCAGAATA ATGCCCCACAG Ggtaagtggt
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FIG._3B

FIG._3C

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3961 catcaatctc caaatccagg ttcatgactc ccctcccata cccagccta
4021 ggctctgttc actcagggga ggaggggaga ctgtactccc cacagaagcc cttccagagg
4081 tccatacca atatccccat ccccactctc ggaggtagaa aggacagat gtggagagaa
4141 aataaaaagg gtgcaaaagg agagaggtga gctggatgag atgggagaga agggggagc
4201 tggagaagag aaagggatga gaactgcaga tgagagaaaa aatgtgcaga cagagaaaaa
4261 aaataggtgg agaaggagag tcagagagtt tgaggggag agaaaaggaa agcttgggag
4321 gtgaagtggg taccagagac agcaagaaag agctggtaga agtcatctca tcttaggcta
4381 caatgaggaa ttgagaccta ggaagaaggg acacagcagg tagagaaacg tggcttcttg
4441 actcccaagc caggaatttg gggaaagggg ttggagacca tacaaggcag agggatgagt
4501 ggggagaga aagaaggggag aaggaaaga tggtgtactc actcatttgg gactcaggac
4561 tgaagtgccc actcactttt ttttttttt tttttgagac aaactttcac ttttgttgcc
4621 caggctggag tgcaatggcg cgatctcggc tcactgcaac ctccacctcc cgggttcaag
4681 tgattctcct gcctcagcct ctagccaagt agctgcgatt acaggcatgc gccaccacgc
4741 ccggctaatt tttgtatttt tagtagagac gggtttcgc catgttggtc agctggtct
4801 cgaactcctg atctcaggtg atccaaccac cctggcctcc caaagtgctg ggattatagg
4861 cgtgagccac agcgcctggc ctgaagcagc cactcacttt tacagaccct aagacaatga
4921 ttgcaagctg gtaggattgc tgtttggccc accagctgc ggtgttgagt ttgggtgcgg
4981 tctcctgtgc tttgcacctg gcccgcttaa ggcatttgtt acccgtaatg ctcctgtaag
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5101 cggttcatcc ttttcattta tacagGGGAT GACCAGAGTC ATTGGCGCTA TGGAGgtgag
5161 acacccaccc gctgcacaga cccaatctgg gaacccagct ctgtggatct cccctacagc
5221 cgtccctgaa cactggtccc gggcgtccca ccgtccgccc acgtcccac cccctcacct
5281 tttctacccg ggttccctaa gttcctgacc taggcgtcag acttcctcac tatactctcc
5341 cacccagGC GACCCGCCCT GGCCCCGGGT GTCCCCAGCC TGCGCGGGCC GCTTCCAGTC
5401 CCCGGTGGAT ATCCGCCCCC AGCTCGCCGC CTTCTGCCCG GCCCTGCGCC CCCTGGAACT
5461 CCTGGGCTTC CAGCTCCCCG CGCTCCCAGA ACTGCGCCTG CGCAACAATG GCCACAGTGg
5521 tgaggggtc tccccgccga gacttgggga tggggcgggg cgcagggaag ggaacggtcg
5581 cgcagtgcct gccggggt ctacggccg gggccggctc acttgcctct
5641 ccctacgcag TGCAACTGAC CCTGCCTCCT CCTGCTAGAGA TGGCTCTGGG TCCCGGGCGG
5701 GAGTACCGGG CTCTGCAGCT GCATCTGCAC TGGGGGGCTG CAGGTCGTCC GGGCTCGGAG
5761 CACACTGTGG AAGGCCACCG AGGtgagcg cggactggcc gagaggggc tgtccttttc
5821 aaaggagcgg ggcggacggg ggccagagac gtggccctct cctaccctcg tgtcctttc
5881 agATCCACGT GGTTCACCTC AGCACCGCCT TTGCCAGAGT TGACGAGCC TTGGGGCGCC
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5941  CGGGAGGCCT  GGCCGTGTTG  GCCGCCTTTC  TGGAGgtacc  agatcctgga  cacccctac
6001  tcccgctt    cccatcccat  gctcctcccg  gactctatcg  tggagccaga  gacccatcc
6061  cagcaagctc  actcaggccc  ctggctgaca  aactcattca  cgcactgttt  gttcatttaa
6121  cacccactgt  gaaccaggca  ccagcccca   acaaggattc  tgaagctgta  ggtccttgcc
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6301  aggtgttcat  tgcagaggaa  acagaatgtg  caaagactca  gaatatggcc  tatttaggga
6361  atggctacat  acaccatgat  tagaggaggc  ccagtaaagg  gaaggatgg   tgagatgcct
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6541  gggattctcc  tgcctcagct  tcctgagtag  ctggggttac  aggtgtgtgc  caccatgccc
6601  agctaatttt  ttttgtatt   tttagtagac  agggtttcac  catgttggtc  aggctggtct
6661  caaactcctg  gcctcaagtg  atccgcctga  ctcagcctac  caaagtgctg  attacaagtg
6721  tgagccaccg  tgcccagcca  cactcactga  ttctttaatg  ccagccacac  agcacaaagt
6781  tcagagaaat  gcctccatca  tagcatgtca  atatgttcat  actcttaggt  tcatgatgtt
6841  cttaacatta  ggttcataag  caaaataaga  aaaaagaata  ataaataaaa  gaagtggcat
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6961  caacacaaag  gtgtatatat  ggtttcctgt  gggagtatg   tacggaggca  gcagtgagtg
7021  agactgcaaa  cgtcagaagg  gcacgggtca  ctgagagcct  agtatcctag  taaagtgggc
7081  tctctccctc  tctctccagc  ttgtcattga  aaaccagtcc  accaagcttg  ttggttcgca
7141  cagcaagagt  acatagagtt  tgaaataata  cataggattt  taagagggag  acactgtctc
7201  taaaaaaaaa  aacaacagca  acaacaaaaa  gcaacaacca  ttacaatttt  atgttccctc
7261  agcattctca  gagctgagga  atgggagagg  actatgggaa  ccccttcat   gttccggcct
7321  tcagccatgg  ccctggatac  atgcactcat  ctgtcttaca  atgtcattcc  cccagGAGGG
7381  CCCGGAAGAA  AACAGTGCCT  ATGAGCAGTT  GCTGTCTCGC  TTGGAAGAAA  TCGCTGAGGA
7441  AGtcagtttt  gttggtctgg  ccactaatct  ctgtgccta   gttcataaag  aatcacccttt
7501  tggagcttca  ggtctgaggc  tggagatggg  ctccctccag  tgcaggaggg  attgaagcat
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7621  ctgcctacag  attgaaaaac  aagcaaaaac  cgccgggcac  ggtggctcac  gcctgtaatc
7681  ccagcacttt  gggaggccaa  ggcaggtgga  tcacgaggtc  aagagatcaa  gaccatcctg
7741  gccaacatgg  tgaaacccca  tctctactaa  aaatacgaaa  aaatagccag  gcgtggtggc
7801  gggtgcctgt  aatcccagct  actcgggagg  ctgaggcagg  agaatggcat  gaaccgggga
7861  ggcagaagtt  gcagtgagcc  gagatcgtgc  cactgcactc  cagcctgggc  aacagagcga.
```

FIG._3D

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7921 gactcctgtc tcaaaaaaaa aaaaaaaaa gaaaaccaag caaaaaccaa aatgagacaa
7981 aaaaaacaag accaaaaaat ggtgttgga gtcaagtctg gagagctaaa
8041 cttttctga gaactgttta tcttaataa gcatcaaata tttaactttt gtaaatactt
8101 ttgttggaaa tcgttctctt cttagtcact cttgggtcat tttaaatctc acttactcta
8161 ctagaccttt taggtttctg ctagactagg tagaactctg cctttgcatt tctgtgtct
8221 gttttgtata gttatcaata ttcatattta tttacaagtt attcagatca tttttttttt
8281 tctttttttt tttttttttt ttttttacat cttagtagaa gacaggggt caccatattg
8341 gccaggctgc tctcaaactc ctgaccttgt gatccaccag cctcggcctc ccaaagtgct
8101 gggattcatt tttctttt aatttgctct gggcttaaac ttgtggccca gcacttatg
d461 atgtacacaca gagttaagag tgtagactca gacggtcttt cttcttcct tctcttcctt
8521 cctcccttcc ctcccacctt cccttctctc ctccttctct ttctcttcct cttgcttcct
8581 caggcctctt ccagttgctc caaagccctg tactttttt tgagttaacg tcttatggga
8641 agggcctgca cttagtgaag agtggtctc agagttgagt tacctttgct tctgggaggt
8701 gaaactgtat ccctataccc tgaagcttta agggggtgca atgtagatga gaccccaaca
8761 tagatccct tcacagGCTC AGAGACTCAG GTCCCAGGAC TGGACATATC TGCACTCCTG
8821 CCCTCTGACT TCAGCCGCTA CTTCCAATAT GAGGGTCTC TGACTACACC GCCCTGTGCC
8881 CAGGGTGTCA TCTGGACTGT GTTTAACCAG ACAGTGATGC TGAGTGCTAA GCAGtgggc
8941 ctggggtgtg tgtggacaca gtgggtgcgg gggaaagagg atgtaagatg agatgagaaa
9001 caggagaaga aagaaatcaa ggctggggctc tgtggcttac gcctataatc ccaccacgtt
9061 gggaggctga ggtgggagaa tggtttgagc ccaggagttc aagacaaggc ggggcaacat
9121 agtgtgaccc catctctacc aaaaaaaccc aacacaaaacc aaaaatagcc gggcatggtg
9181 gtatgcggcc tagtcccagc tactccaaga ggctgaggtg ggaagatcgc ttgattccag
9241 gagttgaga ctgcagtgag ctatgatccc agcactgcct accatcttta ggatacattt
9301 atttatttat aaaagaaatc aagaggctgg atggggaata caggagctgg agggtggagc
9361 cctgaggtgc tggttgtgag ctggcctggg accctgtt cctgtcatgc catgaaccca
9421 cccacactgt ccactgacct ccctagCTCC ACACCCTCTC TGACACCCTG TGGGGACCTG
9481 GTGACTCTCG GCTACACGTG AACTTCCGAG CGACGCGCAGCC CGAGTGATTG
9541 AGGCCTCCTT CCCTGCTGGA GTGGACAGCA GTCCTCGGGC TGCTGAGCC Ggtacagctt
9601 tgtctggttt ccccccagcc agtagtccct tatcctccca tgtgtgtgcc agtgtctgtc
9661 attggtggtc acagcccgcc tctcacatct ccttttctc tccagTCCAG CTGAATTCCT
9721 GCCTGGCTGC TGgtgagtct gccctctccc ttggtcctga tgccaggaga ctcctcagca
9781 ccattcagcc ccaggctgc tcaggaccgc tctcctttc tgcagaacag
9841 accccaaccc caatattaga gaggcagatc atggtgggga ttccccatt gtcccagag
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FIG._3E

```
9901  gctaattgat tagaatgaag cttgagaaat ctcccagcat ccctctcgca aaagaatccc
9961  cccccctttt tttaaagata gggtctcact ctgtttgccc caggctgggg tgttgtggca
10021 cgatcatagc tcactgcagc ctcgaactcc taggctcagg caatcctttc accttagctt
10081 ctcaaagcac tgggactgta ggcatgagcc actgtgcctg gccccaaacg gcccttttac
10141 ttggctttta ggaagcaaaa acggtgctta tcttacccct tctcgtgtat ccaccctcat
10201 ccctggctg gcctcttctg gagactgagg cactatgggg ctgcctgaga actcggggca
10261 gggtgggtgg agtgcactga ggcaggtgtt gaggaactct gcagacccct cttccttccc
10321 aaagcagccc tctctgctct ccatcgcagG TGACATCCTA GCCCTGGTTT TTGGCCTCCT
10381 TTTTGCTGTC ACCAGCGTCG CGTTCCTTGT GCAGATGAGA AGGCAGCACA Ggtattacac
10441 tgaccctttc ttcaggcaca agcttccccc acccttgtgg agtcacttca tgcaaagcgc
10501 atgcaaatga gctgtccctg ggccagtttt ctgattagcc tttcctgttg tgtacacaca
10561 gAAGGGGAAC CAAAGGGGGT GTGAGCTACC GCCCAGCAGA GGTAGCCGAG ACTGGAGCCT
10621 AGAGGCTGGA TCTTGGAGAA TGTGAGAAGC CAGCCAGAGG CATCTGAGGG GGAGCCGGTA
10681 ACTGTCCTGT CCTGCTCATT ATGCCCACTTC CTTTTAACTG CCAAGAAATT TTTTAAAATA
10741 AATATTTATA ATaaaatatg tgttagtcac cttgttcccc caaatcagaa ggaggtattt
10801 gaatttccta ttactgttat tagcaccaat ttagtggtaa tgcatttatt ctattacagt
10861 tcggcctcct tccacacatc actccaatgt gttgctcc
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FIG._3F

FIG._3A
FIG._3B
FIG._3C
FIG._3D
FIG._3E
FIG._3F

FIG._3

FIG._4

FIG._5

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-506  CTTGCTTTTC ATTCAAGCTC AAGTTTGTCT CCCACATACC CATTACTTAA CTCACCCTCG

-446  GGCTCCCCTA GCAGCCTGCC CTACCTCTTT ACCTGCTTCC TGGTGGAGTC AGGGATGTAT
         AP2

-386  ACATGAGCTG CTTTCCCTCT CAGCCAGAGG ACATGGGGGG CCCCAGCTCC CCTGCCTTTC

-326  CCCTTCTGTG CCTGGAGCTG GGAAGCAGGC CAGGGTTAGC TGAGGCTGGC TGGCAAGCAG

-266  CTGGGTGGTG CCAGGGAGAG CCTGCATAGT GCCAGGTGGT GCCTTGGGTT CCAAGCTAGT
                                                                  p53

-206  CCATGGCCCC GATAACCTTC TGCCTGTGCA CACACCTGCC CCTCACTCCA CCCCCATCCT
         Inr                                                     Inr

-146  AGCTTTGGTA TGGGGGAGAG GGCACAGGGC CAGACAAACC TGTGAGACTT TGGCTTCCATC
                                                                  Inr

-86   TCTGCAAAAG GGCGCTCTGT GAGTCAGCCT GCTCCCCTCC AGGCTTGCTC CTCCCCCACC
         AP1                  ***                  p53          AP2

-26   CAGCTCTCGT TTCCAATGCA CGTACAGCCC GTACACACCG TGTGCTGGGA CACCCCACAG
                                                                ...
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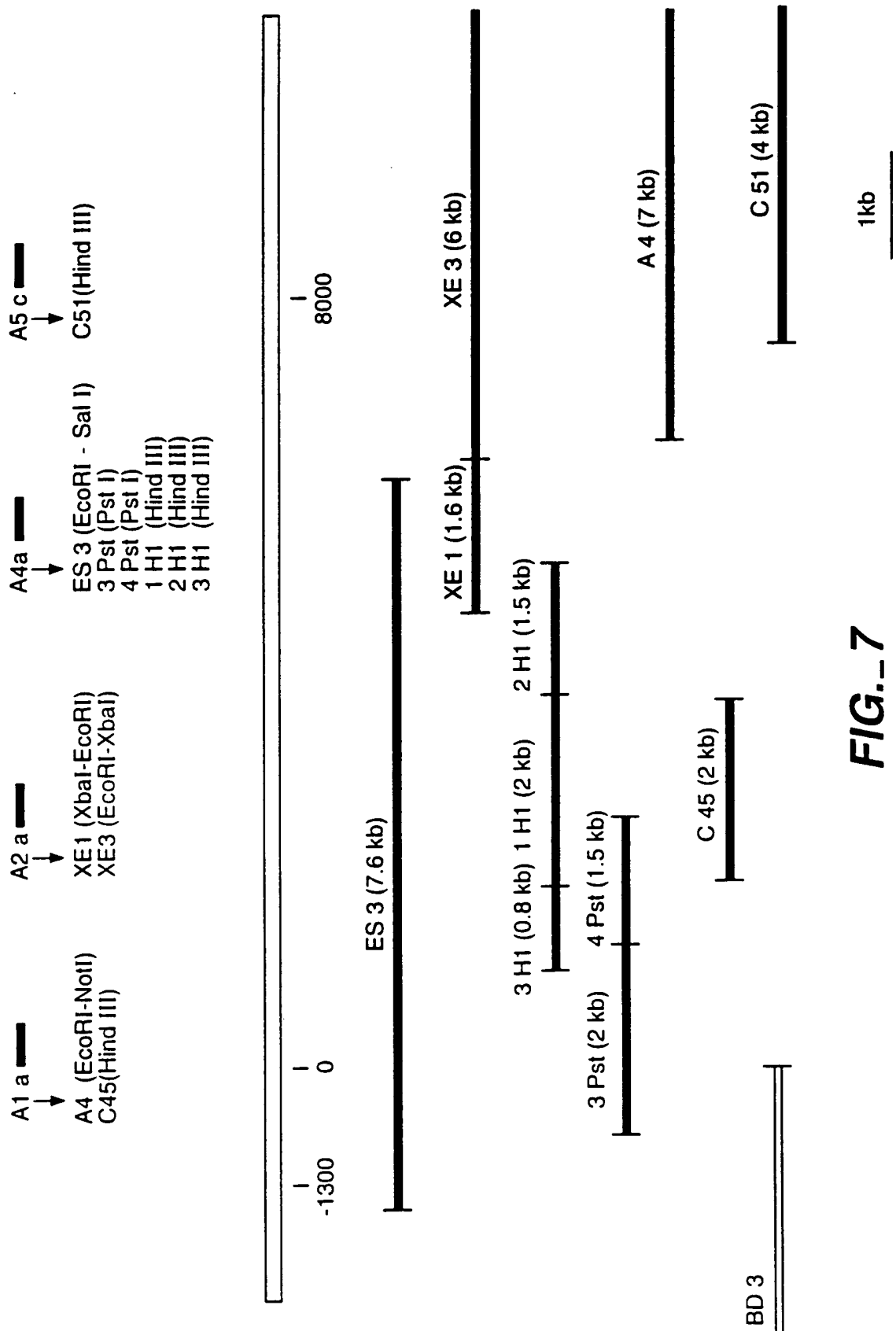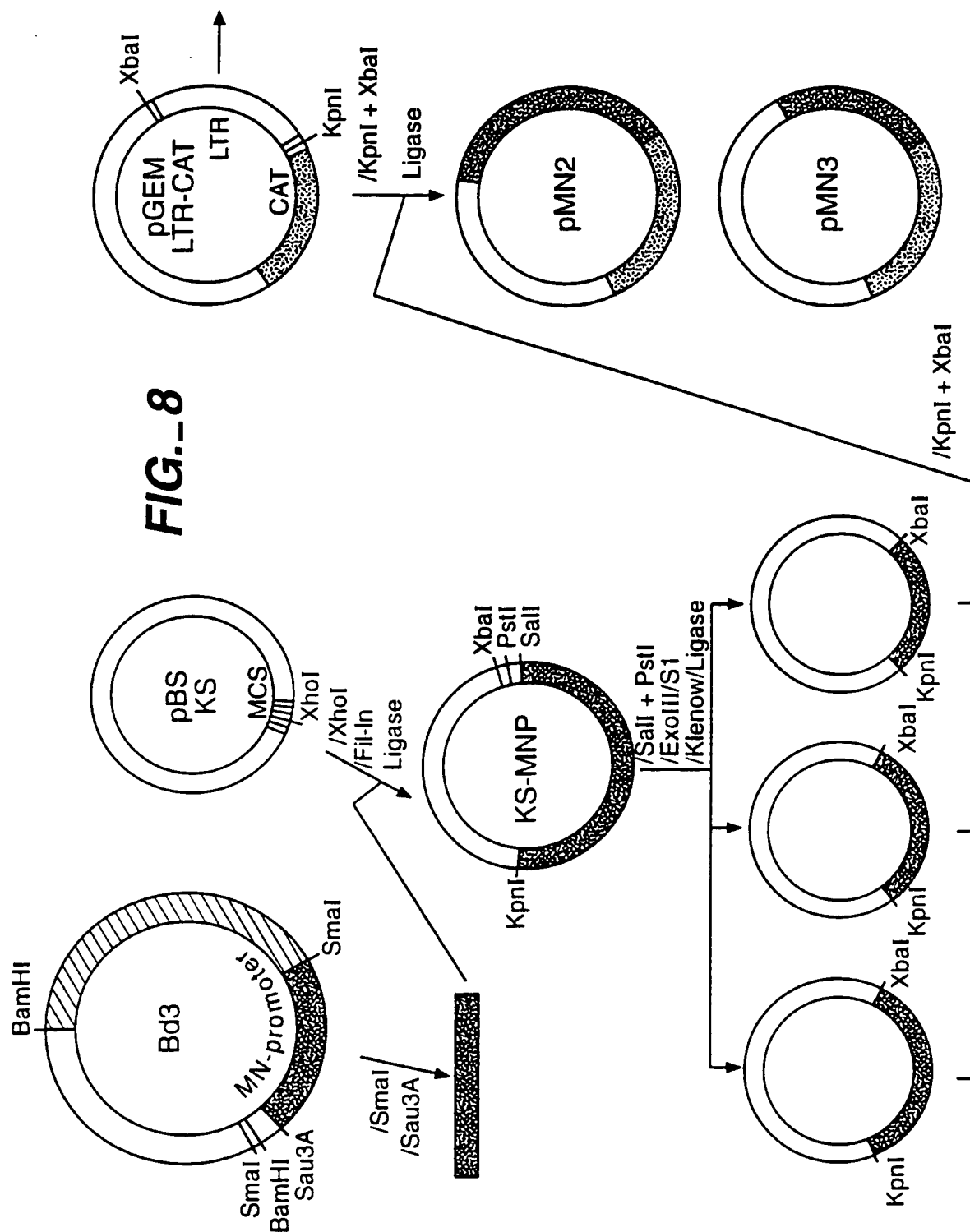FIG._6

FIG._7

FIG._8

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   C12N15/12   C12N1/21   C12N15/62   C12Q1/68   A61K39/00
      C07K14/82   C12N5/16   C12N15/87   C07K16/30   G01N33/50
      A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C12N   A61K   C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 18152 (CIBA CORNING DIAGNOSTICS CORP. AND INSTITUTE OF VIROLOGY) 16 September 1993 cited in the application see the whole document ---	1-14, 16-18, 21-27
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 85, December 1988 WASHINGTON US, pages 8998-9002, M.A.FROHMAN ET AL. 'Rapid production of full-length cDNAs from rare transcripts: Amplification using a single gene-specific oligonucleotide primer' see page 8998 ---	10,28

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 January 1996	16. 01. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (- 31-70) 340-2040, Tx. 31 651 epo nl, Fax (- 31-70) 340-3016	Cupido, M

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VIROLOGY, vol. 187, no. 2, April 1992 ORLANDO US, pages 620-626, S.PASTOREKOVA ET AL. 'A novel quasi-viral agent, MaTu, is a two-component system' see the whole document ---	1-28
P,X	ONCOGENE, vol. 9, no. 10, October 1994 pages 2877-2888, J.PASTOREK ET AL. 'Cloning and characterization of MN, a human tumor-associated protein with a domain homologous to carbonic anhydrase and a putative helix-loop-helix DNA binding segment' see the whole document -----	1-14, 16-18, 21-27

1

| Box I | Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet) |

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:   18,20,21,26
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 18,20,21 and 26 are directed to methods of treatment of, and diagnostic methods practised on the human body the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

| Box II | Observations where unity of invention is lacking (Continuation of item 2 of first sheet) |

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9318152	16-09-93	CA-A-	2131826	16-09-93
		EP-A-	0637336	08-02-95
		JP-T-	7508160	14-09-95
		NO-A-	943344	09-09-94
		US-A-	5387676	07-02-95